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VOLUME 22 NUMBER 9

SEPTEMBER, 1932

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Official Organ of
The American Phytopathological Society

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PHYTOPATHOLOGY

VOLUME 22 NUMBER 9

SEPTEMBER, 1932

PATHOGENICITY OF THREE RED-CEDAR RUSTS THAT OCCUR ON APPLE[1]

PAUL R. MILLER[2]

(Accepted for publication February 15, 1932)

INTRODUCTION

An unusual and severe epiphytotic of quince rust on apples occurred in southern Indiana in 1929 and certain varieties of apples, such as Delicious and Winesap, which had heretofore been considered rust resistant, showed abundant calyx-end infection on the fruit.

There are 3 species of the genus Gymnosporangium infecting apples in Indiana. For clarity and convenience the 3 rust diseases will be referred to as apple rust, caused by *Gymnosporangium juniperi-virginianae* Schw., quince rust, caused by *G. germinale* (Schw.) Kern, and hawthorn rust caused by *G. globosum* Farl. Following are some aids in identifying the 3 fungi:

The color of the aeciospore wall of *Gymnosporangium germinale* is pale yellow as contrasted with the light chestnut brown wall of *G. globosum* and *G. juniperi-virginianae*. The peridium of *G. germinale* dehisces by irregular shredding and does not become revolute. The peridial cells of *G. juniperi-virginianae* become much curved when wet, with the inner and side walls sparsely rugose and the latter with ridges extending halfway across, as contrasted with the smooth inner and side walls of the peridial cells of *G. globosum*. The teliospores of *G. germinale* have carotiform pedicels ranging from 9 to 12 μ in diameter near the spore, as contrasted with the cylindrical pedicels of the other 2 species, 3–5 μ in diameter. The cells of the teliospore of *G. germinale* have only one germ pore per cell, which is

[1] Contribution from the Botany Department, Purdue University Agricultural Experiment Station, LaFayette, Indiana. Thesis presented to the Graduate School of Purdue University in partial fulfillment of the requirements for the M.S. degree.

[2] Junior Pathologist, Bureau of Plant Industry, U. S. Department of Agriculture. Grateful acknowledgment is made to Dr. E. B. Mains for valuable suggestions made during the earlier part of these investigations; to Dr. M. W. Gardner for helpful criticism during the latter part of the work and during the preparation of the manuscript; to Monroe McCown for numerous apple-rust collections; to Professors H. E. Thomas and J. A. McClintock for inoculation material; and to Dr. K. D. Doak for help and suggestions in photography.

located near the apex in the upper cell and near the pedicel in the lower cell, while in the other two species there are two germ pores in each cell located near the septum.

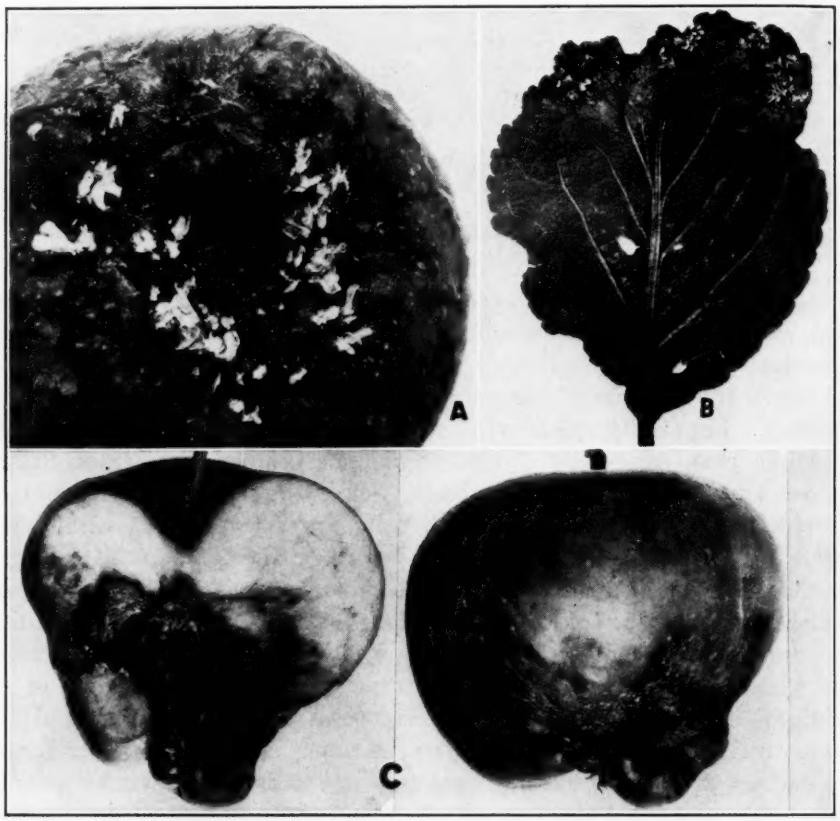

FIG. 1. A. Chimney-like aecia of *Gymnosporangium germinale* on calyx end of Winesap apple that had been stored in a refrigerator (enlarged). B. Leaf of Rome apple, torn across the tip before inoculation. Infection took place only along the torn edge. This indicates that the natural resistance that is acquired at maturity in susceptible varieties can be broken down by wounding. C. Fruit of Delicious variety of apples, showing stunting and malformation caused by calyx-end infection with quince rust (*G. germinale*) and the internal necrosis and cavity formation. Natural infection, 1929.

Quince rust was very destructive on apple fruit in 1929. In the lesions that are typical for Delicious, the tissue is killed while the fruits are small, and, due to the unequal growth, a very dwarfed and misshapen fruit results (Fig. 1, C). The internal tissues are killed to a considerable depth. It is not unusual for these fruits to be cracked open, exposing large cavities in

the flesh. For Winesap the surface of the lesion is usually of a water-soaked dark green color, with larger areas of the fruit surface involved. Aecia are not usually produced. However, occasionally aecia were produced on infected fruits that were stored in a refrigerator (Fig. 1, A).

The symptoms produced by hawthorn rust on apple leaves are very similar to those caused by apple rust except that the lesions are smaller, the aecia are fewer and grouped in the center of the lesion, and the peridial tubes are persistent, are longer than those of apple rust, and dehisce irregularly, never becoming revolute.

On the red cedar the 3 rusts can be differentiated by the following characteristics: At maturity the hawthorn rust gall is reddish in appearance, as contrasted with the greenish-brown apple-rust gall, and instead of pit-like depressions there are on this gall wedge-shape elevated areas, fewer and larger. From each of them a brown tongue-shape gelatinous horn emerges (Fig. 2, C), as contrasted with the slender cylindrical spore horns

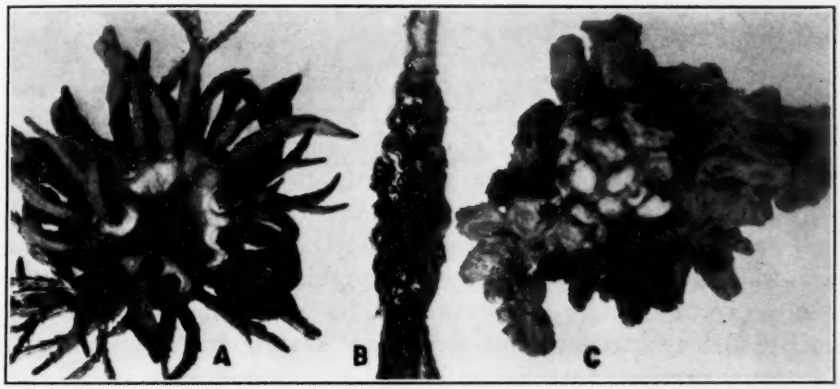

FIG. 2. Gymnosporangium galls on red cedar as they appear with teliospore sori gelatinized and expanded. A. *G. juniperi-virginianae*, showing the cylindrical spore horns. B. *G. germinale*, showing the broad wart-like spore masses. C. *G. globosum*, showing the large tongue-shape spore masses.

of the apple rust (Fig. 2, A). Quince rust produces inconspicuous cylindrical or spindle-shape, roughened swellings on the twigs and limbs of red cedar, which vary from 2 to 60 cm. in length and usually encircle the limb (Fig. 2, B).

VARIETAL SUSCEPTIBILITY IN INDIANA APPLE ORCHARDS

Apple varieties, such as Delicious, Winesap, and Stayman, had been considered rust resistant previous to 1929 in Indiana. In order to determine the resistance or susceptibility of varieties of apples commonly grown

in Indiana, field observations were made from August 15 to 21, 1929, in 18 commercial orchards in southern Indiana. Counts of at least 50 fruits and 100 leaves of each variety were made in each orchard. The records on fruit infection are shown in table 1 and on leaf infection in table 2.

TABLE 1.—*Occurrence of apple rust (Gymnosporangium juniperi-virginianae) and quince rust (G. germinale) on fruit of apple varieties in Indiana orchards, 1929*

Variety	No. of fruits examined	Percentage of fruits infected	
		Apple rust	Quince rust
Jonathan	100	20	0
Rome	200	35	0
Wealthy	150	25	0
Winter Banana	250	10	1
Ben Davis	150	12	0
Winesap	250	0	8
Baldwin	150	0	2
Stayman	150	0	5
Delicious	150	0	15
King David	250	0	2

It will be noted in table 1 that quince rust occurred on fruit of the varieties of Delicious, King David, Winter Banana, Winesap, Baldwin, and Stayman, none of which except Winter Banana were infected with apple rust. A few fruits of Winesap and Delicious were found bearing aecia, while the other varieties bore only pycnia.

Apple rust was severe on the Jonathan, Rome, Wealthy, Winter Banana, and Ben Davis varieties. Some Rome and Wealthy fruits bore aecia, the others only pycnia.

No hawthorn rust was found on the fruit.

As table 2 shows, the varieties Jonathan, Rome, Wealthy, Winter Banana, and Ben Davis were severely infected with apple rust. The lesions were large in most cases, and there were only a few on each leaf. Aecia were produced in abundance on Rome and Wealthy, sparingly on Jonathan, and on Winter Banana and Ben Davis only necrotic flecks with pycnia were produced.

Hawthorn rust was found on the varieties Rome, Jonathan, Baldwin, Winesap, Northwestern Greening, and McIntosh. The lesions were smaller than those caused by apple rust. A few aecia were formed on Rome and McIntosh and only pycnia on the other varieties.

No leaf infection caused by *Gymnosporangium germinale* (quince rust) was found.

TABLE 2.—*Occurrence of apple rust (Gymnosporangium juniperi-virginianae) and hawthorn rust (G. globosum) on leaves of apple varieties in Indiana orchards, 1929*

Variety	No. of leaves examined	Percentage of leaves infected	
		Apple rust	Hawthorn rust
Rome	500	40[a]	12[b]
Ben Davis	200	22[c]	0
Jonathan	300	25[b]	5[d]
Wealthy	500	65[a]	0
Winter Banana	100	10[c]	0
Baldwin	100	0	8[d]
Winesap	200	0	4[d]
Northwestern Greening	100	0	9[d]
McIntosh	100	0	1[b]

[a] Aecia abundant.
[b] Aecia present but not abundant.
[c] Necrotic flecks with pycnia.
[d] Pycnia only.

TELIOSPORE AND BASIDIOSPORE GERMINATION

Red-cedar branches bearing galls caused by *Gymnosporangium juniperi-virginianae* were brought into the greenhouse January 5, 1930, and the cut ends put in water. At the end of 2 weeks these galls showed signs of pushing out spore horns. On January 23 some of these branches were put in a damp chamber and on January 31 spore horns had been pushed out. Portions of these spore horns were put in drops of water on slides and the slides were then placed in a moist chamber. Normal germination of the teliospores occurred.

During the process of spore-horn protrusion some of the leaves on the twigs bearing galls turned yellow and dropped off. Since this yellowing was thought to be due to the excessive drain by the rust on the food supply of the host, sucrose was added to the water, and this seemed to cause the infected cedar twigs to maintain their viability longer. It was necessary to make a fresh cut across the basal end of the limb every few days and to change the solution frequently because of bacterial growth. With this procedure it was possible to obtain an abundance of teliospores for germination and inoculation tests over a period of several months.

Reed and Crabill (13) found 15° C. to be the optimum temperature for the germination of the teliospores of *Gymnosporangium juniperi-virginianae*, while Weimer (15) found it to be 22–25° C.

In order to determine the most favorable temperature conditions for germination of teliospores and basidiospores of the 3 species of rust under

consideration, germination tests in water were made at temperatures ranging from 0 to 36° C. Approximately 5,000 of each of the 2 spore forms of the 3 species of rust were counted. The results are given in table 3. The average percentage germination is given, but with teliospores this can be no more than a general average because there is such a variation in the germinability of these spores, depending upon their maturity, location on the spore horn, and some undetermined factors. The results show that the teliospores of the 3 species of rust will germinate well between 20 and 28° C., with the optimum at about 24° C., while the basidiospores germinated best at 16° C. and fairly well between 12 and 24° C. Three hours at the optimum temperature were necessary for basidiospores to be produced.

TABLE 3.—*Effect of temperature on the germination of teliospores and basidiospores of the three apple rust fungi*

Temperature used	Gymnosporangium juniperi-virginianae		G. globosum		G. germinale	
	Teliospore germination	Basidiospore germination	Teliospore germination	Basidiospore germination	Teliospore germination	Basidiospore germination
°C.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
0	0	0	0	0	0	0
4	.05	0	.5	0	1	0
8	2	.01	1	.4	1.5	.2
12	5	30	3	27	2	8
16	8	65	6	50	4	45
20	52	50	41	41	37	30
24	76	17	60	12	50	16
28	50	1	34	.07	28	.6
32	.2	0	.5	0	.7	0
36	0	0	0	0	0	0

In each of the 3 rust species, an abnormal type of teliospore germination occurred at high temperatures, in which long promycelia were formed but no basidiospores were developed. The basidiospores normally germinate by one or more germ tubes, but, under certain conditions, instead of a germ tube being produced, a sterigma similar to that formed on the promycelium was put forth and on the end of this a secondary basidiospore was formed. The secondary basidiospores appeared to be similar to the primary ones except that they were smaller. Production of such basidiospores could be brought about at will by high temperature and abundant water.

INOCULATIONS OF APPLE VARIETIES AND OTHER AECIAL HOST SPECIES

In May, 1930, inoculations were made on 10 commercial varieties of apples in the orchard, apple seedlings, and potted plants of quince, pear, *Sorbus* sp., and Aronia. Approximately 50 leaves and 25 fruits of each apple variety were inoculated with each of the 3 species of rust. The method used was to suspend a portion of the gelatinized spore horn on a wire about 3 in. above the tissue to be inoculated. The apple leaves were pulled between the fingers and rubbed gently to remove the bloom, so that the water would adhere to the leaf. The leaves or fruits were atomized with water and a piece of cotton soaked in water was wrapped around the stem to insure high humidity in the glassine bag that was put over the spore mass and parts to be inoculated. The glassine bags were closed and fastened with metal clips and were left on about 24 hours.

The results presented in table 4 show that infection with apple rust (*Gymnosporangium juniperi-virginianae*) was secured on the fruits of Rome, Wealthy, Ben Davis, and Grimes and the leaves of Rome, Wealthy,

TABLE 4.—*Inoculations in the orchard with apple rust (Gymnosporangium juniperi-virginianae)*

Variety or species inoculated	Fruit		Leaves	
	No. inoculated[a]	No. infected	No. inoculated[a]	No. infected
Rome	15	12	17	13
Wealthy	10	6	17	17
Ben Davis	9	5	7	2
Grimes	12	2	15	12[b]
King David	27	0	24	15[b]
Jonathan	20	0	16	10
Maiden Blush	12	0	30	0
Delicious	18	0	26	0
Stayman	26	0	23	0
Winesap	26	0	28	0
Pear	.....	.....	10	0
Sorbus sp.[c]	.....	.....	9	0
Aronia[c]	.....	.....	12	0

[a] Number inoculated that did not drop prematurely.
[b] Flecking with no pycnia produced.
[c] Potted plants in greenhouse.

Ben Davis, Grimes, King David, and Jonathan. The symptoms on these infected fruits and leaves were identical in appearance with those occurring in nature. Pycnia appeared about 3 weeks after inoculation. Aeciospores produced on the inoculated fruit and leaves were examined and found to

agree in shape and measurement with the aeciospores of this species of rust. On inoculated Grimes and King David leaves, only necrotic spots were produced and these rarely reached a diameter of one mm. No pycnia were produced in these spots. Inoculated leaves of pear, *Sorbus* sp., and Aronia failed to become infected.

A greater percentage of the inoculated fruit and inoculated leaves dropped prematurely from the varieties that were susceptible than from those that were resistant.

The results presented in table 5 show that no fruit infection was obtained with hawthorn rust (*Gymnosporangium globosum*). Leaf infection with

TABLE 5.—*Inoculations in the orchard with hawthorn rust (Gymnosporangium globosum)*

Variety or species inoculated	Fruit		Leaves	
	No. inoculated[a]	No. infected	No. inoculated[a]	No. infected
Maiden Blush	15	0	30	22[b]
Grimes	18	0	27	20[b]
Ben Davis	14	0	15	12[d]
Jonathan	17	0	13	9[d]
Rome	25	0	12	8[e]
Wealthy	16	0	11	6[e]
Sorbus sp.[c]	.....	.....	16	16[f]
Aronia[c]	.....	.....	35	20[b]
Pear[c]	8	0	15	12[b]
Stayman	14	0	26	0
Winesap	12	0	24	0
Delicious	30	0	26	0
King David	18	0	21	0

[a] Number inoculated that did not fall prematurely.
[b] Necrotic flecks with no pycnia produced.
[c] Potted plants in greenhouse.
[d] Only pycnia produced.
[e] A few aecia produced.
[f] Aecia abundant.

hawthorn rust was obtained on the following apple varieties: Maiden Blush, Grimes, Ben Davis, Jonathan, Rome, and Wealthy, and on the leaves of Sorbus, Aronia, and pear. The symptoms on these infected leaves were identical with those occurring in nature. Spore and peridial tube measurement agreed with the measurements given for *G. globosum*. On the leaves of Maiden Blush and Grimes apples, Aronia, and pear, only necrotic spots without pycnia were produced. On Ben Davis and Jonathan only pycnia

were produced, on Rome and Wealthy a few aecia were produced, and on *Sorbus* sp. there were abundant aecia.

The results of the inoculations with quince rust (*Gymnosporangium germinale*) are given in table 6. Infection with quince rust was secured on

TABLE 6.—*Inoculations in the orchard with quince rust (Gymnosporangium germinale)*

Variety or species inoculated	Fruit		Leaves	
	No. inoculated[a]	No. infected	No. inoculated[a]	No. infected
Delicious	20	13	16	0
Winesap	17	9	13	0
Stayman	8	6	17	0
Wealthy	12	5	22	0
King David	14	0	26	0
Maiden Blush	20	0	24	0
Ben Davis	16	0	15	0
Jonathan	11	0	21	0
Grimes	15	0	35	0
Rome	14	0	20	0
Quince[b]	0	0	15	15

[a] Number that did not drop prematurely.

[b] Potted plant in greenhouse.

the fruits of 4 varieties: Delicious, Winesap, Stayman, and Wealthy. The symptoms were identical with those occurring in commercial orchards on the same varieties in 1929. No visible infection on the apple leaves resulted, but quince leaves were infected. The lesions were confined to the veins, which were swollen and necrotic at the point of infection.

Teliospore material of quince rust obtained from Prof. H. E. Thomas, Ithaca, New York, J. A. McClintock, Knoxville, Tennessee, and from La Fayette, Indiana, in 1931, was used in inoculation tests in the orchard for the purpose of determining whether or not physiologic forms exist in these different localities. Six varieties of apples were used as differential hosts: Delicious and Winesap, since these had previously been found to be very susceptible under Indiana conditions; Stayman and Wealthy (moderately susceptible); and Jonathan and Rome (resistant). The methods of inoculation used were similar to those used in 1930.

The results as indicated in table 7 show that the varieties Delicious, Winesap, Stayman, and Wealthy were susceptible to the inoculum coming from the 3 localities. Jonathan and Rome were resistant, with the exception that 2 Rome fruits inoculated with the spores from Tennessee developed symptoms that were typical for an early stage of quince rust. The fruits

were observed at weekly intervals from the first sign of infection until they were mature, with no difference of symptoms being detected. As in 1930, no visible leaf infection occurred.

TABLE 7.—*Inoculation of apple fruits with Gymnosporangium germinale from New York, Tennessee, and Indiana, 1931*

Variety	New York		Tennessee		Indiana	
	No. fruits inoculated	No. infected	No. fruits inoculated	No. infected	No. fruits inoculated	No. infected
Delicious	25	17	25	15	25	19
Winesap	10	9	10	10	10	8
Stayman	25	14	25	7	25	15
Wealthy	10	6	10	8	10	5
Jonathan	25	0	25	0	25	0
Rome	10	0	10	2[a]	10	0

[a] Two fruits exhibited a dark green, puckered appearance.

EXCISED APPLE LEAVES CARRIED ON SUCROSE SOLUTION

In order to extend the period of time during which inoculation work could be carried out, a modification of the methods used by Mains (12), Clinton and McCormick (1), and Giddings and Leonian (10) has been employed. Excised apple leaves were carried on a 6 per cent sucrose solution in Petri dishes for 2 months, or long enough after inoculation for pycnia and aecia to be produced.

In the earlier experiments a galvanized $\frac{1}{4}$-in. mesh wire was put in the Petri dish, and the petiole of the leaf was inserted through this, so that it was the only part of the leaf in contact with the solution. Later the leaf petioles were inserted through holes in thin layers of cork that were floated in the nutrient solution. The leaves were first washed in running water. The nutrient solution was changed every 5 days, and dead portions of the leaves that were noticed were cut off and removed. The leaves were inoculated in the dishes by atomizing with a basidiospore suspension.

It was found early in this work that the time of day that the leaves were removed from the tree was of great importance. Leaves removed from the tree in the late afternoon remained viable much longer on the sucrose solution than those taken in the morning. This may be accounted for by the probability that leaves taken in the late afternoon contain much starch, while the leaves taken in the morning are low in starch.

With this method of keeping excised apple leaves alive on a sucrose solution, studies were made of the relation of wounds to leaf infection and of heterothallism, as will be presented in the 3 following sections.

RELATION OF WOUNDS TO SUSCEPTIBILITY OF APPLE LEAVES TO APPLE RUST (GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE)

Fulton (8) noted that apple leaves become resistant to rust as they mature. Giddings and Berg (9) observed that in nature mature apple leaves may become infected through insect injuries.

An apple leaf was accidentally torn with a metal clip in the process of bagging an inoculated twig and infection occurred only along this wounded portion. Following this discovery, mature apple leaves of 5 varieties, some of which are considered rust resistant and others of which are susceptible, were used in inoculation experiments with apple rust. Inoculations were made on Rome, Jonathan, Wealthy, and Winesap leaves under field conditions, using the same methods as above, and also on excised Rome, Jonathan, Wealthy, and Delicious leaves carried on sucrose solution in Petri dishes. The leaves were wounded by tearing them slightly, previous to inoculation. Uninjured leaves were inoculated at the same time.

The results as given in table 8 show that no unwounded leaves were infected and that wounded leaves of the susceptible varieties Rome, Jonathan, and Wealthy became infected, while those of the resistant varieties Winesap and Delicious did not. Infection took place only along the wounded portion of each leaf (Fig. 1, B). These results may be interpreted to indicate that the resistance that is shown by mature leaves of susceptible varieties has a morphological basis.

Leaves of the varieties Winesap and Delicious, which are naturally resistant when young, did not become infected when wounded and inoculated, as did those of susceptible varieties, which had acquired resistance because of their maturity.

TABLE 8.—*Relation of wounds to infection of apple leaves with apple rust (Gymnosporangium juniperi-virginianae)*

Location	Variety	Leaves not wounded		Leaves wounded	
		No. inoculated	No. infected	No. inoculated	No. infected
On tree	Rome	20	0	20	12
	Jonathan	32	0	32	15
	Wealthy	16	0	16	13
	Winesap	12	0	16	0
In Petri dish	Rome	6	0	6	4
	Jonathan	6	0	6	2
	Wealthy	6	0	6	5
	Delicious	6	0	6	0

INDICATIONS OF HETEROTHALLISM IN APPLE RUST (GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE)

With methods based on Craigie's work (2, 3, and 4) on heterothallism in *Puccinia graminis,* an attempt was made to determine whether or not apple rust (*G. juniperi-virginianae*) is heterothallic. Tests were carried out under 3 conditions in 1930, viz, potted seedlings growing in the greenhouse, orchard or field conditions, and detached leaves carried in Petri dishes on a sucrose solution.

In the orchard 60 leaves, each bearing only a single pycnial rust lesion, were found and tagged. At the proper time, which was about June 1, the pycnial exudate of 22 of these single lesions was mixed so that each lesion received exudate from several others. Twenty of these lesions had produced aecial sori containing aeciospores about July 20 (Table 9). On the 16 leaves bearing single pycnial lesions on which the pycnial exudate was not mixed only 9 produced aecia. Even though these leaves were not bagged to protect them from insects, it may be significant that 90 per cent produced aecia where the pycnia exudate was mixed and only 56 per cent where it was not mixed (Table 9).

A very dilute spore suspension of basidiospores was atomized on the leaves of potted seedlings, May 15. On certain leaves only a single lesion resulted. These leaves bearing single lesions were bagged separately, and at the proper time, which was about June 8, the pycnial exudate on 12 of those isolated pustules was mixed. All of these lesions had produced aecia bearing aeciospores by July 30 (Table 9). On 7 isolated lesions the exudate was not mixed and no aecia were formed.

A basidiospore suspension was atomized on numerous detached leaves carried on sucrose solution in Petri dishes. On 13 leaves, only a single lesion per leaf resulted. On 8 of these leaves the pycnial exudate was mixed and 6 of these produced aecia (Table 9). Of the 5 leaves on which the pycnial exudate was not mixed only one produced aecia. This one exception may be explained by the possibility that it was a compound lesion derived from two or more basidiospores.

A few monosporidial lesions were obtained by the following methods: Drops of a very dilute spore suspension were put on slides and examined under a microscope. When a drop was found containing less than 5 basidiospores, an attempt was made to withdraw one spore by the aid of a bulb pipette attached to the mechanical stage. The spores remaining in the drop were counted and, if one less spore remained, it was assumed that one spore was in the pipette. The contents of the pipette were released on a leaf and by this method of inoculation a lesion was obtained on each of 8 leaves floated on sucrose solution. These were considered as monosporidial lesions.

The pycnial exudate of 4 of these was mixed and each of these lesions produced aecia (Table 9), while no aecia were formed by the other 4 lesions, where the exudate was not touched.

TABLE 9.—*Tests indicating heterothallism in Gymnosporangium juniperi-virginianae*

	Lesions with pycnial exudate not mixed			Lesions with pycnial exudate mixed		
	Total number	Number with aecia	Percentage with aecia	Total number	Number with aecia	Percentage with aecia
Orchard	16	9	56	22	20	90
Potted seedlings	7	0	0	12	11	92
Detached leaves	5	1[a]	20	8	6	75
Monosporidial pustules	4	0	0	4	4	100

[a] Possibly a compound lesion.

Although the numbers of lesions in these tests are small, the indications are that *Gymnosporangium juniperi-virginianae* is heterothallic in the sense that the transfer of pycnial exudate may be necessary for aecium production in monosporidial lesions.

INDICATIONS OF HETEROTHALLISM IN HAWTHORN RUST (GYMNOSPORANGIUM GLOBOSUM)

Fifty hawthorn leaves, each bearing an immature single pycnial lesion caused by *Gymnosporangium globosum,* were taken from the trees May 12, 1931, and floated on a 6 per cent sucrose solution. At the time of exudation of the pycnia, the exudate was transferred from one leaf to another on 25 leaves, each lesion receiving the exudate from several others. The pycnial exudate on the other 25 leaves was not touched. All leaves were protected from insects. It is interesting to note that the drops of exudate that had received foreign exudate dried down within 24 hours after the process, whereas the unmixed exudate remained for 30 days. By July 17, 1931, 17 of these leaves bearing the mixed exudate had produced aecia with mature spores. The remaining 8 leaves did not bear aecia. Of the 25 leaves bearing the unmixed exudate 4 had produced aecia, the remaining 21 did not.

Two hundred leaves similar to the above were taken from the trees June 4 and were treated in a manner similar to the above. Of the 100 leaves on which the exudate was mixed, 92 per cent had produced aecia by August 15, whereas only 3 per cent of the remaining 100 leaves bearing the unmixed exudate bore aecia.

Ninety leaves, each bearing a single pycnial lesion, were taken from the hawthorn trees June 10 and were put on the nutrient solution. Instead of promiscuous mixing of the exudate, the exudate from a single lesion was transferred to a series of 25 leaves, with special care to sterilize the needle between each transfer. Another series of 40 leaves received the exudate from another single lesion. By August 15, 1931, 22 of the above 25 leaves had produced aecia, and 36 of the 40 had done likewise. Although there is a possibility that the 2 individual lesions from which exudate was transferred were compound (the result of infection by more than a single basidiospore), this is not probable since none of the 25 leaves, each bearing a single lesion with the exudate undisturbed, used as checks, produced aecia. According to Craigie's work (5) on *Puccinia graminis*, one would have expected only about half of these lesions thus treated to produce aecia.

One hundred and fifty hawthorn leaves, each bearing a single pycnial lesion of *Gymnosporangium globosum*, were tagged on May 15, 1931. At the time of exudation of the pycniospores, the exudate was mixed on 75 of these leaves, each lesion receiving the exudate from several other lesions. The exudate of the remaining 75 leaves was not touched. All were unprotected from insects. By August 25, 70 of the leaves bearing the mixed exudate had produced aecia, whereas only 46 of the leaves bearing the unmixed exudate bore aecia.

FACTORS INFLUENCING AECIOSPORE GERMINATION

Heald (11), Reed and Crabill (13), Weimer (15), and Doran (6) experienced difficulty in obtaining good germination of aeciospores of *Gymnosporangium juniperi-virginianae*. Fukushi (7) and Thomas and Mills (14) have found that exposing aeciospores of apple rust to low temperatures increases their germinability.

Various tests were made to determine the factors influencing germination. Aeciospores of *Gymnosporangium juniperi-virginianae* were germinated in drops of sucrose solution ranging from 0.2 to 6 per cent on glass slides in Petri dish moist chambers. The germination with the sugar solution was essentially the same as was obtained in the tap-water checks. Similar results were obtained with an extract of red-cedar leaves. Aeciospores germinated in darkness equally as well as in daylight.

Temperature plays an important part in the germination of aeciospores. Counts of several thousand aeciospores of the 3 species of rust under consideration, germinated at temperatures ranging from 4 to 32° C. at intervals of 4°, have been made. The minimum, optimum, and maximum temperature at which these aeciospores germinate is fairly constant, but the percentage germinating is extremely variable, depending upon the time of the year these germination trials are made and the method of obtaining the

aeciospores from the sorus. If the aeciospores were dusted directly from the leaf onto the slide rather than scraped from the pustule, more uniform results were secured, presumably because only apical, mature spores are thus liberated.

No germination was secured at temperatures lower than 6° C. for *Gymnosporangium juniperi-virginianae* and *G. globosum,* while 9° C. was the minimum temperature for *G. germinale.* The optimum temperature for the germination of aeciospores of *G. juniperi-virginianae* and *G. globosum* was found to be 24° C., while the aeciospores of *G. germinale* germinated best at 16° C. Fairly good germination was secured from *G. juniperi-virginianae* and *G. globosum* from 16 to 28° C., while for *G. germinale* the range was 12 to 20° C. No germination was secured above 32° C. in any of the 3 species.

In August, 1929, apple leaves bearing aecia of *Gymnosporangium juniperi-virginianae* were placed in cheesecloth bags and suspended about 2 ft. from the ground from a limb of a tree, so as to be under natural environmental conditions. Germination tests of these aeciospores were made each month for a year (Table 10). All of these spores were germinated at 24° C., as this had previously been found to be the optimum temperature for aeciospores of *G. juniperi-virginianae.* The percentage of germination was determined by counting 500 aeciospores that were in proper conditions for germination. It is interesting to note that only a small percentage of the aeciospores germinated in August but that the germination percentage increased gradually throughout the fall and winter and reached its highest point in March at 84 per cent. Then, there was a more or less sudden drop, with a few aeciospores germinating even as late as the following August.

In August, 1930, a similar experiment was started and this time aeciospore-germination tests were made twice a month. The same tendency toward increased percentage of germination in the winter was noted (Table 10). On August 1, 4 per cent germination was secured; September 1, 6 per cent; October 1, 14 per cent; November 1, 20 per cent; December 1, 35 per cent; January 1, 46 per cent; February 1, 20 per cent; and March 1, no germination was secured. The cause for the decrease in germination of aeciospores in February and March, 1931, as contrasted with 1930, is not known. However, these months were unusually warm in 1931, and the leaves bearing the aeciospores were invaded by saprophytic organisms.

Tests of a similar nature were made on aeciospores produced in 1931, with results as shown in table 10. The germination percentage increased from 8 in August to 26 in January.

TABLE 10.—*Longevity of aeciospores of Gymnosporangium juniperi-virginianae and the effect of overwintering on their germinability*

1929 Aeciospores		1930 Aeciospores		1931 Aeciospores	
Dates of germination tests[a]	Percentage of germination	Dates of germination tests	Percentage of germination	Dates of germination tests	Percentage of germination
August, 1929	12	August 1, 1930	4	August 1, 1931	8
September	12	" 15	7	" 30	7
October	32	September 1	6	October 30	14
November	25	" 15	15	November 15	22
December	50	October 1	14	" 30	20
January, 1930	50	" 15	15	December 15	22
February	72	November 1	20	January 4, 1932	26
March	84	" 15	35		
April	44	December 1	35		
May	13	" 15	30		
June	7	January 1, 1931	46		
July	1	" 15	45		
August	A few weak germ tubes	February 1	20		
		" 15	8		
		March 1	0		

[a] Approximately the 15th of each month.

Since the aeciospores germinated in March and April, at which time new growth is present on the red cedars, the possibility of spring infection of red cedars is suggested.

SUMMARY

The symptoms produced on the apple fruit by *Gymnosporangium germinale* are very different from those produced by *G. juniperi-virginianae* in that there is no yellowing on the surface, the fruit is misshapen due to the early killing of the tissue, and often there is considerable internal necrosis, rendering the fruit worthless.

The fruits of the following apple varieties were found infected with apple rust (*Gymnosporangium juniperi-virginianae*) in southern Indiana in 1929: Jonathan, Rome, Wealthy, Winter Banana, and Ben Davis. The leaves of Rome, Ben Davis, Jonathan, Wealthy, and Winter Banana varieties were infected with apple rust. Quince rust (*G. germinale*) occurred on fruit of Winter Banana, Winesap, Baldwin, Stayman, Delicious, and King David. The leaves of Baldwin, Rome, Jonathan, Winesap, Northwestern Greening, and McIntosh were infected with hawthorn rust (*G. globosum*).

Successful inoculations were made in the orchard with *Gymnosporangium juniperi-virginianae* on the fruit and leaves of Rome, Ben Davis, Grimes, and Wealthy and on the leaves of Jonathan.

Successful inoculations were made with *Gymnosporangium globosum* on the foliage of Maiden Blush, Rome, Ben Davis, Jonathan, Grimes, and Wealthy and, in addition, on Aronia, pear, and *Sorbus* sp. Only necrotic flecks were produced on Maiden Blush, Grimes, Aronia, and pear. Aecia were produced on Rome, Wealthy, and *Sorbus* sp.

Successful inoculations were made with *Gymnosporangium germinale* on the fruit of Delicious, Winesap, Stayman, and Wealthy varieties, and on quince leaves. The fruit symptoms produced resembled those resulting from natural infection.

The leaves of varieties easily infected with *Gymnosporangium juniperi-virginianae* in the young stage with production of pycnia and aecia are not naturally infected at later stages of maturity. When however, mature leaves of such varieties (Rome, Jonathan, Wealthy) were wounded, inoculation was successful and pycnia and aecia were produced. On wounded leaves of resistant varieties, such as Winesap and Delicious, inoculation was not successful.

For use in inoculation or for studies of heterothallism apple leaves removed from the tree at the end of the day were found to remain alive on a 6 per cent sucrose solution in Petri dishes for two months, which was sufficient time for aecia to develop.

In both *Gymnosporangium juniperi-virginianae* and *G. globosum*, the majority of lesions resulting from infection by single basidiospores do not produce aecia. When the pycnial exudate from such lesions was mixed, the majority of the lesions produced aecia. This indicates that these rusts are heterothallic.

The optimum temperature for teliospore germination for *G. juniperi-virginianae*, *G. globosum*, and *G. germinale* was found to be 24° C.; for the basidiospores of the 3 species 16° C.; the aeciospores of *G. juniperi-virginianae* and *G. globosum* germinated best at 24° C.; while the optimum temperature for *G. germinale* was found to be 16° C.

At high temperatures (32 to 36° C.) the three rust species exhibited an abnormal type of teliospore germination in which long promycelia with no basidiospores were produced. Secondary basidiospores were formed under conditions of high temperature and abundant water.

Aeciospores of *Gymnosporangium juniperi-virginianae* lived over winter and germinated well in March and April. Some aeciospores germinated one year after collection. Aeciospores scraped out did not germinate as well as those dusted out.

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THE PROPERTIES OF PLANT VIRUSES FROM DIFFERENT HOST SPECIES[1]

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Not only are the properties of plant viruses of general interest in relation to their bearing on the nature of a virus and on the control of the diseases that they cause, but they may serve in whole or in part as a means of isolation, differentiation, and classification of certain groups of viruses. Previous investigations have, in general, shown that the properties of a particular plant virus, such as that of ordinary tobacco mosaic from tobacco, are fairly definite and constant characters. While some variation has been claimed, it has not ordinarily been beyond that of normal biological variation or such as may be explained either through differences in material and methods or through experimental error.

Very little reliable information appears to exist, however, relative to the influence of different host species on the properties of any particular virus. If the source of the inoculum markedly affected the properties of a virus, this would naturally have some bearing on the nature and control of a virus disease and would perhaps complicate the value of property studies for purposes of differentiation.

The problem of the influence of the host species on the properties of a virus appeared, therefore, to be sufficiently important to warrant our making a fairly detailed investigation, such as is presented in this paper, even though the evidence, as it accumulated, indicated that the virus properties were not influenced in any material way by the host species in which the virus was propagated and from which it was extracted.

EARLIER INVESTIGATIONS

The studies by Allard (1, 2) and others on the ordinary tobacco-mosaic virus and those by Doolittle (3) on the cucumber-mosaic virus on the respective host plants showed that these viruses possessed fairly definite properties. When these properties are compared, it is evident that they are distinctly different in most particulars, although the significance of this fact was not at first realized and their similarity rather than their difference was emphasized. During this period there was a tendency to assume that apparently different viruses diseases were due to essentially the same causal agent and to account for the differences in properties and behavior on the basis of the

[1] Cooperative experiments with the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture. Also supported in part by the University of Wisconsin Research Fund.

particular host concerned. This belief, which may be illustrated by Walker's (11) conclusion that it is possible to change the property of a virus radically by transferring it to another host, was quite generally accepted. McKinney (10), considering this question from a somewhat different angle, suggested that the rapid loss of the infectious property of expressed cucumber-mosaic virus may be due in part to the nature of the cucumber-plant fluids and that the thermal death point of the tobacco-mosaic virus depends on the nature of the plant extract. More recently, on the other hand, specific plant viruses have been reported in increasing numbers with little or no evidence supporting the suggestion that a virus is a labile entity.

MATERIALS AND METHODS

The method of experimentation used in this investigation consisted simply in inoculating the specific viruses used to a variety of susceptible host species. When infection on these plants was evident, extracts were made and treated as desired, following which inoculation was made to a series of tobacco plants to determine the effect of the treatment on the virus. A considerable number of minor difficulties may naturally arise in performing trials of this sort, but, in general, these will be passed over as having no significant bearing on the results or conclusions.

Four viruses were selected for the study, namely, the ordinary tobacco-mosaic virus (*tobacco virus 1* (7)), the cucumber-mosaic virus (*cucumber virus 1* (7)), the tobacco-ring-spot virus (4), kindly furnished by Dr. S. A. Wingard, and the "spot-necrosis virus," now recognized as a combination of two viruses (9). Particular attention was paid, however, to the "mottle" virus constituent of the combination.

The host plants used as sources of inoculum were primarily of the family Solanaceae, chiefly for the reason that these were for the most part susceptible to all the viruses used. In a few cases representatives of other plant families were used, *i.e.*, cucumber and Martynia. In general, however, the range in type of hosts used is believed to be sufficient for the purposes of the investigation. Tobacco, *Nicotiana tabacum* L., was always used more or less as a standard or control. Two other species of this genus were also frequently used, namely, *N. rustica* L. and *N. glutinosa* L. Other host species commonly used were tomato, *Lycopersicon esculentum* Mill; pepper, *Capsicum annuum* L.; ground cherry, *Physalis pubescens* L.; petunia, *Petunia violacea* Lindl.; and eggplant, *Solanum melongena* L. *Nicandra physaloides* (L.) Pers., *Solanum atropurpureum* Schr., *Datura stramonium* L., *Cucumis sativus* L., *Apium graveolens* L., *Martynia louisiana* Mill., *Solanum nigrum* L., *S. miniatum* Bernh., *S. tuberosum* L., *S. carolinense* L., *Spinacia oleracea* L., and other species were less frequently included. Difficulties in securing infection or good symptoms with some of the viruses on

certain hosts account for the apparent omission of what might otherwise prove interesting comparisons. In other cases a lack of seed of particular hosts resulted in a failure to secure adequate repetition of trials. In general, however, it was planned to run at least 3 separate trials with each virus from each host, inoculating 5 tobacco plants for each test.

The method of inoculation used was at first the cotton-needle prick and scratch method. Later, the rubbing method of Holmes (6) was used, especially in the dilution trials, since a higher percentage of infection could be secured by this method at high dilutions. The trials are only comparative, however, and usually the only question involved was whether the virus extract was or was not infectious following a given treatment. The percentage of infection secured was not usually regarded as of particular interest in the present trials.

The properties of the virus may naturally include a considerable number of characteristics, but for the present studies only 4 properties were selected for investigation, namely, the thermal death point, longevity *in vitro*, tolerance to dilution, and resistance to certain chemicals.

The methods used in the determination of these properties have been as simple as possible, first because they were intended, in any case, only to be comparative, and secondly because any attempt at too close refinement in technique might serve not only to complicate the results but also to make the usefulness of the methods too laborious to be practical.

The thermal-death-point determinations were made by placing about 2 cc. of freshly extracted juice in thin, stoppered test tubes (care being taken not to drop any extract on the sides of the tube) and placing these in an agitated constant-temperature water bath for 10 minutes, after which the tubes were rapidly cooled in water. With certain methods of inoculation, such as the leaf-mutilation method on potato, it is impractical to work with much less than 2 cc. of inoculum. It has, therefore, seemed advisable not to work with too small quantities of material in determinations of this type.

The aging tests were made by placing the extracts in stoppered test tubes for the required lengths of time in a darkened drawer in a greenhouse where the temperature usually ranged between 80–90° F. The dilution tests were made in the ordinary manner with distilled water. The chemical tests were made by combining one part of virus extract with one part of the chemical at double the strength to be tested. The virus was consequently diluted only ½, and by addition of one part of virus extract the chemical was brought to the required strength. The duration of the treatment can naturally be varied to any desired time but should not, of course, exceed the length of time the virus will live *in vitro*. In the present investigation, treatments of 1, 24, and 48 hours were tested.

EXPERIMENTAL RESULTS

Tests were performed on the thermal death point of all 4 viruses included in the experiments. The tobacco-mosaic virus was not, however, included in the aging tests on account of its extreme longevity, which rendered it impracticable to conduct the work. Trials on tolerance to dilution and resistance to chemical action were limited to the tobacco-mosaic virus and the cucumber-mosaic virus, as being sufficient for illustrative purposes. The results of the experiments are summarized in tables 1 to 11. Some incompleteness in the tables may appear to exist, brought about largely by the advisability of occasionally shifting the range of the treatments given.

Thermal death point. The thermal death point or inactivation point of ordinary tobacco-mosaic virus from tobacco is generally accepted as being close to 90° C. for a 10-minute treatment. Some variation is to be expected as a consequence of various circumstances, even though the conditions of the trials are made as constant as possible. According to McKinney (10), higher dilutions of the virus may lower the thermal death point several degrees; however, our tests have been carried on with undiluted extracts. On account of the results of previous investigations, we were at first somewhat puzzled by the failure of the tobacco-mosaic virus from tobacco to become inactivated at 90° C. in some of our trials, but we are convinced that part of this behavior may be attributed to minor modifications in technique, such as the use of the rubbing method of inoculation, and to normal biological variation.

The experiments were planned so as to submit the virus from the various hosts to temperatures well above and below the suspected thermal death point, at 5° C. intervals. If the virus in question was inactivated at a lower temperature or unaffected at a higher temperature, evidence of host influence on the thermal death point would be shown. However, it was found desirable to shift the range of temperatures in some cases, so the results presented in the tables are not always strictly comparable.

The results of studies on the influence of the host species on the thermal death point of the tobacco-mosaic virus are shown in table 1. In no case did the virus withstand a temperature of 95° C., although in several trials it withstood exposure at 90° C. Exposure at 85° C. apparently had little effect on the virus from most species tested, though in the case of *Solanum miniatum, S. atropurpureum, S. melongena,* and *Martynia louisiana* considerable inactivating action apparently occurred at this temperature. Hence, it may be concluded that in these and in most other species tested the thermal death point for the tobacco-mosaic virus is below 90° C. but that certain host species concerned may vary the thermal death point as much as 5° C. Relatively, this is not a variation of sufficient magnitude to

TABLE 1.—*The influence of host species on the thermal death point of the tobacco mosaic virus*[a]

Source of inoculum	Temperature °C. (10 min.)				Inoc. controls
	80	85	90	95	
Nicotiana tabacum (tobacco)	30/30	80/74	80/48	65/0	85/85
" *rustica*	20/20	15/15	20/8	—	20/20
Nicandra physaloides	5/5	20/17	5/0	20/0	20/20
Capsicum annuum (pepper)	10/5	25/15	10/0	15/0	25/25
Petunia violacea (petunia)	—	15/15	—	15/0	15/15
Physalis pubescens (ground cherry)	5/5	20/13	5/2	15/0	20/20
Lycopersicon esculentum (tomato)	10/10	30/29	15/1	20/0	30/30
Solanum atropurpureum	—	20/3	—	20/0	20/20
" *melongena* (eggplant)	10/10	15/2	15/0	—	15/15
" *nigrum*	5/5	5/3	5/0	—	5/5
" *miniatum*	15/15	25/5	25/0	—	25/25
Martynia louisiana	15/14	15/1	15/0	—	15/15

[a] In this and in succeeding tables, upper figure represents number of plants inoculated; lower figure, number of plants infected.

be considered significant in the present state of our knowledge concerning plant viruses.

The thermal death point of the cucumber-mosaic virus, according to Doolittle (3), is 70° C., although his data indicate that the virus apparently survived heating at 70° for 10 minutes in some cases, though it did not survive 75°; consequently, the thermal death point would probably be somewhat above 70°. Our own experience with the thermal death point

of this virus, first from tobacco (7) and later from other hosts (Table 2), indicates that it behaves rather erratically, probably on account of its sensitivity to various circumstances other than the host species from which the inoculum is taken.

TABLE 2.—*The influence of host species on the thermal death point of the cucumber-mosaic virus*

Source of inoculum	Temperature °C. (10 min.)					Inoc. control
	55	60	65	70	75	
Nicotiana tabacum (tobacco)	10/6	25/23	20/11	10/0	15/0	20/19
" *rustica*	10/1	15/3	10/0	10/1	10/0	10/10
" *sylvestris*	—	5/0	5/0	5/0	—	5/5
" *quadrivalvis*	—	5/0	5/0	5/0	—	5/5
" *glutinosa*	15/5	15/2	10/0	10/0	—	15/5
Petunia violacea (petunia)	10/10	25/4	25/0	10/0	15/0	25/24
Physalis pubescens (ground cherry)	—	15/0	15/0	—	15/0	15/11
Lycopersicon esculentum (tomato)	5/0	15/5	20/2	5/0	15/0	20/14
Solanum melongena (eggplant)	10/0	10/0	5/0	5/0	—	10/8
" *nigrum*	5/3	10/4	10/1	10/0	—	10/8
Cucumis sativus (cucumber)	—	15/0	—	—	15/0	15/10
Apium graveolens (celery)	5/5	5/2	—	5/0	—	5/5

It became advisable, therefore, to run some trials at a temperature as low as 50° C., although usually the range was 55 to 75°. In no case did the virus survive 75° C., and in only one questionable case did it survive 70° C. It was, however, frequently inactivated partially or entirely at 65 and 60° C., but this behavior did not appear to be correlated with any particular host

when sufficient trials were run to warrant satisfactory conclusions. A variability between 60 to 70° C. for the thermal death point of the cucumber-mosaic virus may be expected, therefore, although actually it probably lies closer to the latter than the former figure.

When the experiments with the tobacco-spot-necrosis-virus complex (potato-rugose mosaic) were first undertaken, it had not yet been definitely ascertained that two separate viruses were concerned. The two types of symptoms were, therefore, referred to as the "spot-necrosis" form and the "mottle" form, and it was recognized that the former was much more susceptible to inactivation by various means than was the latter. In previous determination, the thermal death point of the mottle form, based upon extractions from tobacco and potato, was found to be 70° C., while the spot-necrosis form was destroyed at temperatures of 5 to 10° lower (8).

TABLE 3.—*The influence of host species on the thermal death point of the spot-necrosis viruses*[a]

Source of inoculum	Temperature °C. (10 min.)				Inoc. control
	55	60	65	70	
Nicotiana tabacum (tobacco)	15/8(3)[a]	15/0(15)	15/0(14)	15/0	15/15
" *rustica*	10/10	10/0(10)	10/0(10)	10/0(2)	10/10
" *glutinosa*	5/1(4)	10/0(8)	10/0(8)	10/0	10/10
Petunia violacea (petunia)	15/10(4)	15/0(15)	10/0(8)	15/0	15/15
Datura stramonium (jimson weed)	5/0(5)	10/0(5)	10/0(4)	10/0	10/0(10)
Lycopersicon esculentum (tomato)	5/0(5)	10/0(10)	10/0(10)	10/0	10/10
Solanum tuberosum (potato)	10/5(5)	10/0(9)	10/0(8)	10/0(1)	10/10
" *miniatum*	5/2(3)	10/0(10)	10/0(5)	10/0(1)	10/10

[a] Mottle form in parentheses.

The range of temperatures used in the present study was 55 to 70° C. In table 3 we have attempted to show the separate action on each virus by placing the results for the mottle form in parentheses. This table again

serves to indicate that the thermal death points of the viruses have not been altered appreciably when these are taken from several distinct solanaceous host plants.

Our previous experience with the tobacco-ring-spot virus was limited, and little was known of its properties at the time this investigation was undertaken. Recently, however, Henderson and Wingard (5) have reported the thermal death point of this virus to be between 60 and 70° C., and this agrees, in general, with our results. The data shown in table 4 indicate that it is never killed at as low a temperature as 60° C. but that

TABLE 4.—*The influence of host species on the thermal death point of the tobacco-ring-spot virus*

Source of inoculum	Temperature °C. (10 min.)				Inoc. control
	55	60	65	70	
Nicotiana tabacum (tobacco)	10/10	10/10	10/6	10/0	10/10
" *rustica*	5/5	10/10	5/5	10/0	10/10
" *glutinosa*	5/5	10/10	10/1	10/0	10/10
" *sylvestris*	—/—	5/4	5/0	5/0	5/5
Petunia violacea (petunia)	15/15	15/15	10/10	10/0	15/15
Datura stramonium (jimson weed)	10/9	10/8	10/2	10/0	10/9
Solanum melongena (eggplant)	5/5	15/4	10/0	15/0	15/9
" *miniatum*	5/5	10/5	10/0	10/0	10/10

from some hosts it may be destroyed at 65° C. and that it never survived 70° C. The thermal death point, therefore, may perhaps eventually be narrowed down to a 5° range (*i.e.*, about 63 to 68° C.).

The results of the tests with these viruses on several different host species seem to show that the source of the inoculum has not caused any radical change in the thermal death point. Variations that occur might in some cases be attributed to the host species, but we do not regard them in any case as of sufficient magnitude to support the contention that this property is not sufficiently constant to warrant its utilization in a comparative

analysis of the viruses themselves. Such minor variations as do occur may, perhaps, prove to be of value for differentiation purposes where special distinctions need to be drawn.

Resistance to aging in vitro. Doolittle (3) aged the expressed juice from several species of cucurbits affected with cucumber mosaic and found that the extract was never infectious after 3 to 5 days and in most cases lost its virulence within 24 to 48 hours. Reference to table 5 shows that

TABLE 5.—*The influence of host species on resistance to aging in vitro of the cucumber-mosaic virus*

Source of inoculum	Time of aging				Inoc. controls
	1 hour	1 day	2 days	3 days	
Nicotiana tabacum (tobacco)	15/15	15/1	15/0	—	15/15
" *sanderae*	—	5/5	5/0	5/0	5/5
" *sylvestris*	—	5/3	5/3	5/1	5/5
" *glutinosa*	20/5	10/3	15/1	10/3	20/20
Petunia violacea (petunia)	15/12	20/3	20/2	5/0	20/20
Physalis pubescens (ground cherry)	15/2	15/0	15/0	—	15/13
Lycopersicon esculentum (tomato)	15/10	15/0	15/0	—	15/9
Solanum melongena (eggplant)	—	—	10/1	5/0	10/8
" *nigrum*	5/5	10/3	10/1	10/0	10/7
Cucumis sativus (cucumber)	15/10	15/0	5/0	—	15/12
Apium graveolens (celery)	5/5	5/5	5/0	—	5/5

similar results have been secured with this virus from various species of the Solanaceae, as tested in the present experiments. *Physalis pubescens* shows a tendency to shorten the life of the virus and *Nicotiana glutinosa* and *N. sylvestris* Speg. & Ca. perhaps a tendency to lengthen it, though in

the latter case the number of plants used was not sufficient to warrant a satisfactory conclusion. The evidence of any material influence of host species on the length of life of this virus *in vitro* is practically *nil* when compared with the remarkable longevity of the ordinary tobacco-mosaic virus.

The tobacco-ring-spot virus seems somewhat more resistant to aging than the cucumber-mosaic virus, since it is still fairly infectious in tobacco extract after 3 and 4 days aging at room temperatures (Table 6). Hen-

TABLE 6.—*The influence of host species on the resistance to aging in vitro of the tobacco-ring-spot virus*

Source of inoculum	Time of aging (days)						Inoc. control
	1	2	3	4	5	6	
Nicotiana tabacum (tobacco)	25/24	25/23	25/11	25/5	10/1	10/0	25/25
" *rustica*	15/10	15/15	20/11	15/5	15/5	10/6	20/20
" *sylvestris*	5/2	5/0	5/0	—	—	—	5/5
Datura stramonium	10/9	10/3	10/0	10/0	—	—	10/9
Solanum miniatum	10/8	10/5	10/0	5/0	5/0	5/0	10/10
" *melongena* (eggplant)	15/8	20/4	20/1	15/2	20/0	15/1	20/9
" *carolinense*	10/8	10/5	5/3	10/0	5/0	5/0	10/7
Spinacia oleracea (spinach)	10/3	10/1	10/0	10/0	10/0	10/0	10/1
Petunia violacea (petunia)	5/5	5/0	5/1	5/0	—	—	5/5

derson and Wingard (5) reported that this virus lost its infectivity in extract after 1 day at room temperature. This discrepancy indicates, perhaps, that in aging tests the viruses should be stored at known constant temperatures. With this virus as with the former, there is but little indication of any material change in longevity when the virus is taken from different hosts. The ring-spot virus from *Nicotiana rustica,* to be sure, shows rather strikingly longevity as compared with the same virus from

spinach, though this difference would, no doubt, be reduced if a larger number of trials were made.

TABLE 7.—*The influence of host species on the resistance to aging in vitro of the spot-necrosis viruses*[a]

Source of inoculum	Time of aging (days)							Inoc. control
	2	4	8	12	14	16	20	
Nicotiana tabacum (tobacco)	5/5	10/7(3)[a]	20/6(11)	20/2(10)	20/2(8)	10/1(5)	5/0(4)	20/20
Nicotiana rustica	——	——	10/1(7)	10/0(7)	10/0(6)	10/0(6)	5/0(4)	10/10
" *glutinosa*	——	5/3(2)	15/2(11)	15/0(6)	15/4(3)	10/4(6)	5/1(4)	15/15
Petunia violacea (petunia)	——	——	10/0(4)	10/0(2)	10/0(3)	10/0(4)	10/0(1)	10/10
Lycopersicon esculentum (tomato)	5/4(1)	10/6(3)	10/6(3)	5/0(1)	10/1(3)	5/2(1)	——	10/7(3)
Solanum melongena (eggplant)	5/0(2)	5/0(5)	10/0(5)	10/0(6)	10/0(4)	5/0(4)	5/0(2)	10/0(8)
Solanum tuberosum (potato)	5/3(2)	10/0(7)	15/0(5)	15/0(7)	15/0(0)	5/0(4)	5/0(0)	15/14(1)

[a] Mottle form in parentheses.

In table 7 are shown the results of aging the tobacco-spot-necrosis-virus combination. If we consider the spot-necrosis form alone, the data presented indicate some variation in behavior, especially between extracts from tobacco and from potato, these results agreeing with similar observations made earlier (8). The greatly reduced percentages of infection obtained with extracts from tobacco and other hosts after aging for several days, as compared with the controls, suggest, however, that too much significance should not be attached to this variation. The spot-necrosis form was not recovered from eggplant, even in the inoculated controls; consequently, the results secured with this host are not comparable with those secured with the other species for this form of the disease.

With regard to the mottle form alone, the data for which are shown in parentheses in table 7, there appears to be no evidence of any significant influence of the host species on the longevity *in vitro*.

There is admittedly room for further study of this property under more carefully controlled conditions than have obtained in the present experi-

TABLE 8.—*The influence of host species on tolerance to dilution of the tobacco-mosaic virus*

Source of inoculum	Dilution of virus					
	None	1 to 100	1 to 1,000	1 to 10,000	1 to 100,000	1 to 1,000,000
Nicotiana tabacum (tobacco)	10/10	10/10	10/10	10/10	10/9	10/9
Capsicum annuum (pepper)	5/5	5/5	10/10	10/10	10/8	10/9
Petunia violacea (petunia)	15/15	15/15	20/20	20/20	20/20	20/20
Lycopersicon esculentum (tomato)	15/15	15/15	15/15	15/15	15/14	15/12
Solanum nigrum	5/5	5/5	10/10	10/10	10/10	10/3
" *miniatum*	5/5	5/5	10/10	10/10	10/10	10/7
Martynia louisiana	10/10	10/10	10/10	10/10	10/9	10/10

ments. The increasing evidence that environmental conditions during aging of an extract have considerable influence on longevity may account for much of the variation in the present data, since no special effort was made to maintain uniformly constant conditions. In relation to the present studies, however, it was not believed that a repetition of these trials under constant environmental conditions was necessary.

Tolerance to dilution. It might be assumed that tolerance to dilution would be more markedly affected by the host species in which the virus develops than would any other property, since both multiplication and adsorption of the virus particles might conceivably be readily influenced. However this may be, there is little evidence in support of this assumption in our dilution trials with the tobacco-mosaic virus (Table 8). Extracts from all hosts, when diluted to 1 to 1,000,000, gave good infection, except those from *Solanum nigrum*, which showed a fairly marked falling off at this dilution. Still higher dilutions should, perhaps, have been studied, since with the rubbing method of inoculation the limit at which infection may take place is probably considerably higher than 1 to 1,000,000. However, the data presented illustrate the significant point that this virus does not lose its infectivity at high dilutions regardless of the host species in which it develops.

TABLE 9.—*The influence of host species on tolerance to dilution of the cucumber-mosaic virus*

Source of inoculum	Dilution of virus					
	None	1 to 100	1 to 1,000	1 to 10,000	1 to 100,000	1 to 1,000,000
Nicotiana tabacum (tobacco)	10/10	10/10	10/9	10/5	15/2	15/0
" *rustica*	15/15	15/13	15/4	15/1	15/1	15/0
Petunia violacea (petunia)	15/15	15/10	15/4	15/2	15/0	15/0
Nicandra physaloides	10/10	10/10	10/8	10/2	10/0	10/0
Lycopersicon esculentum (tomato)	10/8	10/10	10/8	10/0	10/1	5/0
Solanum melongena (eggplant)	10/8	10/5	15/6	15/2	15/0	
" *nigrum*	5/5	5/5	10/3	10/4	10/0	10/0
Apium graveolens (celery)	10/10	10/10	10/8	10/3	10/1	10/0

The cucumber-mosaic virus is incapable of causing infection at as high dilutions as the tobacco-mosaic virus, even if the rubbing method of inoculation is used. Doolittle (3) secured infection at 1 to 10,000 from mosaic-cucumber extracts, using another method of inoculation. It is interesting to note that, while the present trials (Table 9) show some instances of infection at 1 to 100,000, a marked falling off occurred at 1 to 10,000. The rubbing method of inoculation, therefore, does not appear to be so effective with this virus, in comparison with other methods, as in the case of the tobacco-mosaic virus. Considering the number of plants used in the dilution tests with the cucumber-mosaic virus, the results secured with the various hosts are very similar.

Resistance to chemicals. The high resistance of the tobacco-mosaic virus to various chemical treatments was demonstrated by Allard (1). Doolittle (3) made similar though less extensive trials with the cucumber-mosaic virus and came to the conclusion that the tobacco-mosaic virus was apparently slightly more resistant to chemicals than the former. Extensive studies of a wide range of chemicals on a variety of viruses would, no doubt, be productive of some very interesting results. Our previously re-

TABLE 10.—*The influence of host species on the resistance to chemicals of the tobacco-mosaic virus*

Source of inoculum	Nitric acid 1 to 200		Ethyl alcohol 50%		Inoc. control
	24 hours	48 hours	24 hours	48 hours	
Nicotiana tabacum (tobacco)	25 25	15 15	25 25	15 15	25 25
Capsicum annuum (pepper)	20 18	15 15	20 20	15 15	20 20
Petunia violacea (petunia)	15 15	15 15	15 15	15 15	15 15
Physalis pubescens (ground cherry)	15 13	15 15	15 15	15 15	15 15
Nicandra physaloides	15 15	15 15	5 5	15 14	15 15
Lycopersicon esculentum (tomato)	20 20	15 15	20 20	15 15	20 20
Solanum atropurpureum	5 5	15 15	5 5	15 15	15 15
" *nigrum*	5 5	5 5	5 5	5 5	5 5

ported limited trials on this subject (7), together with the results secured in the present studies (Tables 10 and 11), show rather conclusively that the tobacco-mosaic virus and the cucumber-mosaic virus are very different with respect to their resistance to the action of nitric acid and alcohol and that they cannot be placed in the same category in this respect. In order to arrive at the approximate lethal points for each virus, entirely different concentrations of chemicals or times of exposure would need to be used. However, it is again believed that the data presented in tables 10 and 11 are sufficient to indicate that the host species from which the respective viruses were obtained have neither tended to increase nor decrease the resistance to the chemicals used and that this particular property as well remains strikingly constant in this respect.

DISCUSSION

The studies on the influence of host species on the properties of plant viruses were at first undertaken with some hesitancy as to their value in view of the rapidly increasing evidence that plant viruses are specific entities that cannot be changed over from one known form to another.

TABLE 11.—*The influence of host species on the resistance to chemicals of the cucumber-mosaic virus*

Source of inoculum	Nitric acid 1 to 200		Ethyl alcohol 50%		Inoc. control (1 hour)
	1 hour	24 hours	1 hour	24 hours	
Nicotiana tabacum (tobacco)	30/2	30/0	30/4	30/0	30/25
" *rustica*	10/1	10/0	10/0	10/0	10/9
" *glutinosa*	5/0	5/0	5/0	5/0	5/5
Petunia violacea (petunia)	15/0	10/0	15/0	15/0	15/14
Physalis pubescens (ground cherry)	15/0	15/0	15/0	15/0	15/13
Lycopersicon esculentum (tomato)	20/0	20/0	20/0	20/0	20/19
Cucumis sativus (cucumber)	15/0	15/0	15/0	15/0	15/8

However, as long as any belief remained that host species could radically change the properties of a virus or that these properties cannot be satisfactorily compared, such beliefs constituted a challenge to the progress of any scheme of differentiation and classification of plant viruses utilizing properties as one criterion.

The experimental results shown in tables 1 to 11 present evidence as to the constancy of these properties in their broader aspects. It may be pointed out that Walker's (11) conclusions were drawn at a time when the specificity of viruses was not generally recognized and that McKinney's (10) conclusions with respect to hosts species are apparently not based on data of his own but rather on Walker's results. Until further data are presented to support the contention that virus properties are not reliable comparative criteria of the specific viruses themselves, it seems logical to utilize these simple and convenient means for purposes of differentiation, together with any other characteristics that may be of value. Refinement in technique is, of course, desirable and may be quite necessary for finer distinctions between the ever-increasing numbers of viruses now being recognized.

The results of the present investigation suggest that property studies might quite as well be carried on with one susceptible plant species as another and still be comparable with previous work. However, there are

some manifest advantages in the use of certain hosts for such studies. Consequently, in working with new or previously described viruses found on new or old hosts, it may still be advisable to transmit the virus, whenever possible, to some convenient and common host plant for purposes of property studies.

In conducting the experimental trials described, many interesting features other than those already discussed were noted, which merit further study. Some of these are closely related to the problem under consideration and are worthy of mention in the present connection.

The influence of the "source of inoculum" on the relative ease with which a virus may be recovered from a given host has been a moot point for some time. Previous experience had indicated that certain hosts showing distinct symptoms as a result of inoculation with a virus failed partially or completely to yield infectious extracts in the ordinary manner. In certain cases this behavior apparently may be influenced by such factors as age and vigor of the host plants, environmental conditions, or localization of symptoms. Following repeated trials, evidence of complete failure to secure infection from certain hosts has been gradually breaking down. However, some evidence of partial failure may be noted in the present investigation in the inoculated controls with the cucumber-mosaic virus (Tables 2, 5), and the ring-spot virus (Table 6), although this behavior apparently has not affected the properties of the virus as such.

Not only may the ease of recovery of a virus be influenced to a considerable extent, but the incubation period and the intensity of the symptoms are apparently affected in some cases, although only in rare cases did we apparently secure actual modification (attenuation) of the virus itself, namely, with passage of tobacco mosaic through Martynia. Aside from these cases, the influence of host species on symptoms appeared to be temporary, and it is not at all clear that these influences were actually due to the host species involved rather than to the condition of the host or virus itself.

Judging from the differences in relative susceptibility of the various hosts used, approaching immunity in some cases, it may logically be reasoned that the viruses are actually subjected to very different circumstances within the various hosts, and the fact that they remain as constant as they do argues strongly against any theory that the virus results in whole or in part from any activity or product of the host itself but that it is rather an entity quite foreign to the host concerned.

SUMMARY

1. The thermal death point, resistance to aging *in vitro*, dilution tolerance, and the resistance to certain chemicals of the viruses of ordinary

tobacco mosaic, cucumber mosaic, tobacco ring spot, and tobacco spot necrosis (potato-rugose mosaic) have been compared in extracts from several species of host plants.

2. The experimental results show that the host species in which the viruses developed did not radically influence the constancy of the properties of each virus. Some minor influences were noted that are hardly to be regarded as of sufficient magnitude to be of actual significance.

3. The contentions that have previously been made in the literature that plant viruses may be fundamentally changed by the host plant affected and that the properties of viruses cannot be adequately studied in a comparative way with the ordinary technique are consequently not supported by the results secured.

4. It is believed that the properties of artificially transmissible plant viruses offer one of the most convenient and reliable criteria for their isolation, differentiation, and classification.

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A CENTRALIZED SCAB-SPRAY SERVICE

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Pioneer work in spray-information service was started in New York State during the World War.[1] Food production and conservation were imperative. To aid in this noble cause an experiment in better timing of apple-scab sprays was initiated in a few New York counties. From this successful venture, the idea of giving timely spray information to growers has spread to other important eastern apple-producing States.

Under a decentralized system it was extremely difficult for the spray-service supervisor to keep in constant touch with the field men, especially when some men traveled over several counties. It was equally difficult for the county agent to keep in touch at all times with the field man of his section. Sometimes growers would call the county agent and ask whether it was time to spray. Perhaps, the grower would add that his neighbor was spraying or that his neighbor, who might be in a different county, had received word to spray. This was an embarrassing situation for the county agent, who, with many projects to supervise, might not know what to recommend and then would call headquarters. Not knowing the stage of bud development or what the field man had said, headquarters frequently was helpless. The result was constant confusion during the early season. An additional handicap was that experienced field men were not always available for a few months' service, and there was great risk in putting an inexperienced man in charge of such work. With a decentralized plan, the success of a spray service in any given locality depends to a large extent upon the judgment of the local field man.

Improvements in communication equipment have greatly aided in making a centralized spray service possible. Telephone, telegraph, and radio all play an important rôle. Since 1925 the Ohio spray service has been expanded and modified to fit more nearly Ohio conditions.[2] The greatest concentration of fruit in Ohio is found in those counties that are located on the rim of the State and that border the Ohio River or Lake Erie. However, in each of the 88 counties there are some commercial growers whose major incomes are derived from the sale of fruit. How adequately to advise such growers was a real problem in the early days of the spray

[1] Barrus, M. F. The organization of a special extension service in New York State. U. S. Dept. Agr. Off. Coop. Ext. Work. Ext. Path. 1(2): 4–10. 1923. (Mimeographed.)

[2] Appreciation is hereby acknowledged to Dr. H. C. Young, of the Ohio Agricultural Experiment Station, and his staff and to Dr. W. G. Stover, of the Ohio State University, for initiating and aiding in developing the spray service.

service. The fact is such scattered growers were entirely ignored. The shift in Ohio has been gradual from a decentralized system involving the use of 8 or 10 highly trained men for a relatively short time in the spring to a centralized plan. In this latter plan, one individual makes recommendations for the time of application of the various sprays for the entire State, in so far as diseases are concerned. Consultation with other patholo-

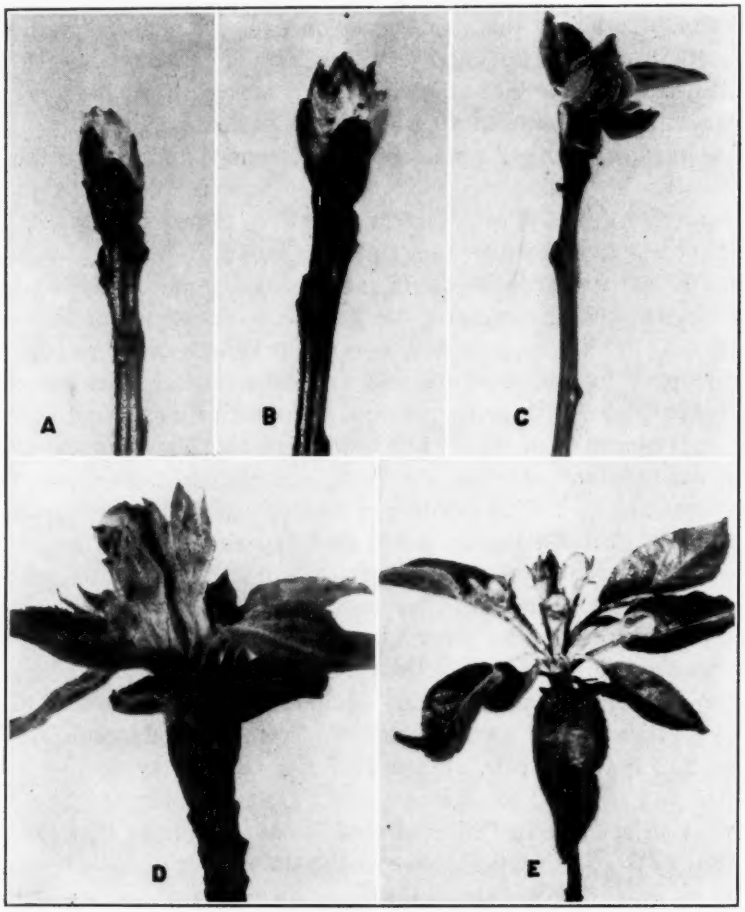

FIG. 1. Stages in apple-bud development. A. Silver bud. B. Delayed dormant. C. Early prepink. D. Prepink. E. Pink.

gists is naturally frequent. Under this plan it is possible for every fruit grower in the State to receive timely spray information.

Three factors are taken into consideration before a decision upon the time of application is reached. The first is the stage of fruit-bud develop-

ment; the second, the percentage of mature ascospores of the scab fungus; and the third, the probable weather for the next 2 or 3 days.

Information on fruit-bud development is obtained from distant points by telegraph or telephone. About 25 cooperators send telegraphic reports to the central office 3 times each week, on Monday, Wednesday, and Friday mornings. If development is extremely rapid, a daily wire is sent to the central office. A small booklet containing 8 colored pictures illustrating the different stages of bud development, from silver bud to full pink, is furnished each cooperator (Fig 1). With this as a guide and following the terminology used in the booklet, the stage of bud development of 3 to 5 common commercial varieties is wired to the central office. In addition to bud development, information on the number of hours of continuous

FIG. 2. Areas used in apple-scab radio broadcasting.

rainfall and on the progress of spraying in the community is included in the messages. These reports begin when the earliest blooming variety approaches the delayed dormant stage and cease when the latest blooming variety is in the late pink stage (Fig. 1, B and E, respectively).

Following is one of the many messages received last spring from a fruit-grower cooperator: "April 26, 1931: 'Duchess and early varieties pink. Baldwin early pink closely approximating pink. Rome late prepink. Cold weather has brought the buds very close together. Rained continuously last 48 hours.' Signed C. H. Stokes."

The State is divided into 17 broadcasting areas, the boundary lines of which was determined by observing fruit-bud development (Fig. 2). These boundaries are changed as our knowledge of bud development increases. In each broadcasting area there is at least one cooperator. In

some areas there are several. In most of these areas, some cooperators are fruit growers, some are county agricultural agents, and two are vocational agricultural teachers. In the hilly section it is necessary that a cooperator state both hill and valley conditions. Along Lake Erie, development at different distances from the lake must be given. As rapidly as these reports are received they are transferred to a large State outline map that has been divided into broadcasting areas.

A second set of cooperators—in some cases the same individuals who send telegraphic reports—send scabby apple leaves of the previous season to the central office 3 times a week. Many growers include samples of new buds with the old leaves. These leaves are usually received and examined within 30 hours after they are collected. A supply of self-addressed, stamped envelopes is furnished each cooperator.

After boiling the leaves in a concentrated solution of either sodium or potassium hydroxide to remove most of the opaque organic matter, the perithecia of the scab fungus can be speedily and accurately counted. From each collection one or two leaves, without boiling, are placed in a humid chamber and the spore discharge noted on the following day. In the fall of the year each collaborator is instructed to collect several hundred scabby apple leaves and place them on the ground under an apple tree near his home with a piece of wire netting over them in order to be certain that he will be able to find old scabby leaves in the spring.

A record of ascospore development is kept, for each cooperator. The details of this record are shown in table 1. The most important columns are the percentage of mature spores, or nearly mature, and, after petal fall, the percentage discharged. By receiving leaves regularly triweekly an accurate record of ascospore development for each area of the State is obtained. It will be noted that a few ascospores were mature for a month before any discharge was observed. There were no heavy rains in that section of the State from which the leaves were received during that interval. It has frequently been observed that a light rain will not cause discharge of many ascospores if the old leaves have been thoroughly dried for a week or 10 days immediately before the rain, even though the spores appear to be mature. From the 18th to the 28th of April, continued rains occurred and 60 per cent of the spores were discharged.

Three samples of leaves are received on successive days about one week after petal fall. From these samples an indication of the potential load of spores still to be discharged can be obtained. The higher this percentage, the greater is the need for 10 days' to 2 weeks' spray.

Special weather forecasts giving the probable temperature, precipitation, and wind velocity for the next 2 or 3 days are received daily. All 3 of these factors must be taken into consideration when making spray recommendations.

TABLE 1.—*Ascospore development record, 1930*

Name of grower—Dean Clippinger Address—Van Wert, Ohio County—Van Wert

No. of sample	Date collected	Date examined	No. of leaves	No. perithecia examined	No. perithecia crushed	Perithecia full-size	Protoplasm granular	Asci cut out	Hyaline spores	2-cell hyaline spores	Mature spores	Spores discharged
	Mar.	Mar.				*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
7	15	18	4	70	15	30	70	30	20	5	1–3	0
12	18	19	5	75	16	35	65	35	20	6	1–2	0
34	24	25	5	90	15	30	70	30	12	10	2–3	0
		Apr.										
49	31	1	4	80	18	40	60	40	15	5	4–6	0
	Apr.											
64	3	5	5	75	8	30	70	30	15	8–10	3–4	0
83	5	7	3	95	10	40	60	40	14	12	6–7	0
99	7	8	4	90	15	40	60	40	12	15	8–9	0
150	9	10	4	100	25	70	30	70	10	20	10	0
164	16	17	3	95	20	70	30	70	7	18	38	0
180	18	19	4	90	25	72	28	72	6	15	38	5
208	21	22	5	100	15	75	25	75	3	14	35	10–15
215	25	26	10	95	15	90	10	90	3	14	32	25–30
		May										
225	28	1	4	95	20	92	8	92	4	12	20	60
		Apple trees in bloom										
	May											
251	15[a]	16	9	70	20		Spore discharge in				20	65
254	16	17	9	85	24		moist chamber—some				25	65
260	17	19	10	75	18		" " —abundant				20	65

[a] Seven, 8, and 9 days, respectively, after petal fall, to aid in determining the necessity of a 10–14 days' spray.

To illustrate clearly the details of the spray service, the procedure during a typical April day is chronologically recorded.

Shortly after 8:00 a. m. telegraph and telephone messages begin to arrive. By 9:45 all the reports have been received and have been transferred to a State outline map. About 10:00 a. m. the special weather forecast is received. Further consultation by telephone with the local Weather Bureau or pathologists at Wooster may be required. The percentage of mature ascospores in various sections of the State is noted. A radio talk of about 600 words is then prepared, giving the bud development for the various areas and suggestions for spraying or for withholding the spray. Differences in varietal bud development and variations in hill and valley orchards must be considered. By 11:15 the talk is given to the local telegraph operator, who has his lines cleared and is directly connected with the simplex machine in the distant radio station of WLW, Cincinnati, and with WTAM, Cleveland. As rapidly as he sends the message it is received on the ticker tape in Cincinnati and Cleveland. About 15 minutes are required to transmit the message, the accuracy of which depends entirely upon the sender. The simplex machines are electrically controlled, and, by checking the message as it emerges from the sending machine, errors are reduced to a minimum.

The county agricultural agents located in Cincinnati and Cleveland put the spray message on the air about 11:50 a. m. and 12:25 p. m., respectively. The actual time of broadcasting is arranged with the broadcasting-station staff each spring, and every fruit grower is notified by mail when to tune in. From 3 to 5 talks are given each week in addition to the weekly summary talks given over WEAO, the local University station.

Preceding the time for each spray or group of sprays, a mimeographed letter is sent to each fruit grower on the county agents' mailing lists. These letters are prepared jointly by the extension plant pathologist and the extension entomologist. They are sent to the county offices in bulk. After affixing his signature, the county agent mails one to each grower. These letters contain all the information necessary for applying a spray, except the time of application for those sprays that are timed by radio. When the critical scab period has passed the extension entomologist takes charge of the radio and broadcasts weekly until about July 1. Information on the severity of scab or on the necessity of applying Bordeaux for blotch and Brooks' spot is included in his weekly broadcasts.

Since human nature is fundamentally alike the world over, some Ohio growers usually feel that certain sprays are not correctly timed for their particular orchards. Granting that they may have some grounds for their views, nevertheless one usually finds that there are other contributing factors within the grower's control to account for their spray failures. To check on these points certain growers were formerly requested to spray a

portion of their orchard exactly as directed by the spray service. The grower was given a chart upon which was kept an accurate record of his spraying activities. Counts on insect and disease injuries were made by a representative of the extension service at harvest time. The results were so gratifying that more and more growers requested forms upon which to keep spray records until at the present time not all the orchards can be visited in the fall where such records are kept. To be sure, not all these growers will follow in detail every recommendation made by the spray service. This is not desired, because not all growers have exactly the same problems.

The average of the results in the orchards where spray recommendations were generally followed are given in table 2. The average of 95.75 per cent

TABLE 2.—*Four years' results of apple spraying where spray-service recommendations were followed*

Year	Number of orchards	Times sprayed	Percentage of scab	Percentage of other diseases	Total percentage	Percentage disease-free fruit
1928	21	6.1	4.5	0.4	4.9	95.1
1929	27	7.1	5.26	0.45	5.71	94.29
1930	98	6.0	2.37	0.01	2.38	97.62
1931	94	5.5	2.0	2.0	4.0	96.0
Average		6.2	3.53	0.71	4.25	95.75

disease-free fruit is fairly high for a group of approximately 100 farmers. The commercial grower cannot afford to have absolutely clean fruit. The last several per cent costs too much to produce. If 95 per cent of the fruit is absolutely disease-free, at least 97 per cent is commercially disease-free. Insect injuries average about the same as those for diseases, so that the commercial grower would have 90 per cent of his fruit free from blemishes. Culls due to disease or insect injuries would not exceed 7 per cent.

There are several advantages in using the radio for making spray recommendations. Different sets of conditions can be outlined and the intelligent grower can then apply those recommendations that best fit his needs. Studying his own orchard problems, he can frequently save one or two sprays during years of light scab-fungus infection and can apply more sprays during severe seasons. It makes it possible for the intelligent grower to produce a clean crop year after year by using a minimum number of sprays.

When necessary, radio talks may be given every day for 5 minutes or longer and an opportunity is afforded for teaching the "why" of spraying in addition to the "when" and the "how." If the growers see the reason

behind the recommendation, they will be more faithful in their efforts. Sometimes weather conditions are uncertain and it is impossible to accurately forecast a rain 3 or even 2 days in advance. Growers can be warned that conditions are uncertain. By outlining what will happen in their orchards if the impending rain occurs on unprotected foliage and then by mentioning the other side of the story, that if it does not rain the spray is wasted, the growers can make their own decisions based upon experience. It is one of the purposes of extension teaching to aid the farmers in making correct decisions. On the following day, when more accurate information on the threatening rain is available, growers located in sections where fruit buds are unprotected can be urged to spray or to withhold the application. By no other means than by the radio is it possible to reach all interested growers every day. It is almost equal to a visit to their individual orchards.

Time is an all-important factor in apple-scab control. A few minutes mean many dollars to 4,000 fruit growers. They are quick to take advantage of the radio, as is evidenced by the letters and telegrams they send to the central office during the spray season. Many request a reply the following day by radio. If radio spray service is to be successful, the broadcaster must be familiar with orchard operations and the station must be powerful enough for the talks to be received without fail on farm sets. Ohio is fortunate in this respect in that two 50,000-watt stations are located in opposite ends of the State.

One of the criticisms of any spray-information service is that year after year the same type of information is outlined for the growers. This must necessarily be the case because growers do not have the physical equipment with which to study ascospore development; even if they had, only a few would interpret the facts correctly. They can observe fruit-bud development accurately in their own orchard, and this they must do in order to apply sprays intelligently. Three-day weather forecasts are not available to them and such forecasts require considerable interpretation before using.

The spray service in Ohio has taught the fruit growers that sprays must be applied promptly. The service has had a marked influence on thoroughness of spraying and has made it necessary for the individual to study critically his own problems. By striving to cover their orchards in as short a time and as thoroughly as possible many orchard men have effected economies in spraying as high as 50 per cent. Since spraying is approximately 30 per cent of the total growing cost of an apple crop, this is a sizable item. These advantages coupled with the increased control obtained by better timing have made the spray service very popular.

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VERTICILLIUM WILT OF COTTON IN MISSISSIPPI

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In the fall of 1930 a hitherto unreported disease of cotton was discovered in Mississippi. This discovery was entirely fortuitous and occurred in connection with operations designed with regard to an entirely different trouble. A collection of 19 cotton stalks, showing internal discoloration supposedly symptomatic of Fusarium wilt, made on some experimental plots at the Delta Branch Experiment Station at Stoneville, Mississippi, on being cultured, gave no growth of *Fusarium vasinfectum*, as was expected. On the other hand, each and every stalk gave rise to a pure culture of a species of Verticillium that agreed in all essential morphological characters with the descriptions of *V. alboatrum* R. and B. These cultures were compared with one of that organism secured from C. D. Sherbakoff and were found to be in agreement. Sherbakoff's culture was made from cotton from Lake County, Tennessee, in 1928.

Verticillium alboatrum was first recorded on cotton in 1914 by Carpenter (1), who found it causing wilt symptoms on 2 plants at Arlington, Virginia. No descriptions or experimental data were recorded by Carpenter, but he expressed the opinion that *V. alboatrum* might be responsible for some of the damage to cotton that is now attributed to *Fusarium vasinfectum*. In 1918 (2) he briefly reported successful inoculation of cotton plants with Verticillium secured from okra. In 1921 (3) and 1922 (4) Bewley reported similar success on cotton with Verticillium from tomato.

In 1928 Sherbakoff[3] briefly reported the discovery of the disease in cotton on the gumbo types of soil of the Mississippi River bottom in Tennessee. In 1929 (5, 6), in other brief articles, he records its discovery in the same type of soil on the opposite side of the Mississippi River, in Arkansas. Aside from citations of the above-mentioned papers by Rudolph (7, p. 306) in 1931 in his extensive treatise on Verticillium hadromycosis, no other reference to a Verticillium on cotton has come to the writers' attention.

Published descriptions of the disease as it appears on cotton are very meager and unsatisfactory. Bewley (3, 4) merely reports that the plants that he inoculated were stunted and that desiccation of the leaves occurred

[1] Plant Pathologist, Mississippi Agricultural Experiment Station and Mississippi State Plant Board.

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[3] Sherbakoff, C. D. Wilt caused by *Verticillium alboatrum*. U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. Sup. 61: 283–284. 1928. (Mimeographed.)

35 days after inoculation. Carpenter (2) reported that 80 per cent of 20 plants inoculated showed symptoms of wilt in 14 days. He further states that Fusarium wilt and Verticillium wilt are indistinguishable except by cultural methods. Sherbakoff (5), in his brief article in PHYTOPATHOLOGY, makes an attempt to distinguish between the two, but his descriptions are contradictory. In an acquaintance with and observation of the disease over a period of 2 years, the writers have been unable to discover any difference in symptoms that has been consistent enough to allow them to distinguish between the 2 wilts in the field without further recourse to laboratory methods for confirmation. At present the writers can only state that the symptoms of the Verticillium wilt as observed are identical with those of the wilt caused by *Fusarium vasinfectum* as those of the latter organism are manifested on soil types on which the Verticillium has been observed. A further study of the symptomatology of the Verticillium wilt that is at present contemplated may, however, reveal differences.

Although the Verticillium wilt was first discovered in Mississippi in the fall of 1930, the plots on which it was found have been used continuously since 1928 for the testing of varietal resistance of cotton to wilt. The wilt against which resistance was being tested was supposedly that caused by *Fusarium vasinfectum*. This testing work was begun by D. C. Neal in the spring of 1928 and was turned over to the senior writer in the fall of that year. Heavy, artificial inoculation of the soil at planting time with cultures of *F. vasinfectum* grown on wheat bran and mixed with sand for distributional purposes was resorted to by Neal in 1928 and by the writers in 1929 and 1930. It was, therefore, assumed that the large amount of wilt that developed was due to that organism. This supposition was rendered still more plausible by the fact that the culture of *F. vasinfectum* that was used in making the soil inoculum for the plots and that the senior writer found in stock when he took over the project bore a label indicating that it had been isolated by Neal from a plant in those same plots the preceding year.

However, examination of the records for the 3-year period from 1928 to 1930 revealed that varieties of cotton that were showing resistance to Fusarium wilt in other parts of the State were not responding similarly in these plots. The idea at once occurred that this difference might be due to the presence in these plots of a strain of *F. vasinfectum* that differed in pathogenicity from those strains present in the plots in other parts of the State. It was to test this idea that diseased stalks were collected and isolations made in 1930, with the resultant discovery of *Verticillium alboatrum* as the causative agent rather than a new pathogenetic strain of *F. vasinfectum*. In addition to these 19 isolations made in 1930, 40 more were made from diseased plants in these plots in 1931 and from not a single one

has a culture of *F. vasinfectum* been secured. By far the greater number have yielded pure cultures of *V. alboatrum*. It would seem, therefore, that *F. vasinfectum* has not been able to persist in this soil.

Careful actual wilt counts are available from these plots for the past 4 years, 1928 to 1931. The average infection for these years is as follows: 1928, 62.82 per cent; 1929, 80.00 per cent; 1930, 35.53 per cent; 1931, 17.66 per cent. It will be seen that there is throughout this period a wide yearly variation in the percentage of actual wilt infection as revealed by counts at the end of the season. The higher percentages in 1928, 1929, and 1930 may possibly be accounted for by the artificial soil inoculation with *Fusarium vasinfectum* that was made in those years. No artificial inoculation was resorted to in 1931. Had this inoculation had an appreciable effect, however, one would have expected it to be revealed in an increased wilt count on the varieties that elsewhere in the State have shown a high degree of susceptibility to Fusarium and, conversely, in a lower wilt percentage in the varieties resistant to Fusarium. Careful analysis of the figures for those years does not yield an impression that they are the result of a combined effect of the Verticillium naturally present and the Fusarium that was introduced. The lower infection in 1930 may have been due to the very severe and extended drought of that year, serving to restrict the activities of one or both of the pathogens. The still lower infection count in 1931 may have been due to the dying out of the Fusarium possibly present in previous years; or, on the other hand, it may have been the result of environmental factors unfavorable to the Verticillium. Sherbakoff[4] states that it is the opinion of the growers in Lake County, Tennessee, where he first discovered the disease, that it occurs there irregularly, or about once in 3 or 4 years. The uniformity of infection on all varieties in 1931 when 60 isolations failed to reveal any Fusarium and in the preceding 3 years leads one to believe that Verticillium alone is chiefly responsible for the wilt on these plots.

Forty varieties of cotton in all have been tested for wilt resistance on these plots in this 4 year period, 1928 to 1931. Not all, however, have been in the test for the full period. In table 1 is shown the average actual wilt infection as recorded at harvest time for each variety that was represented in the tests for at least 2 years.

The varieties were planted in 107 foot rows and in 4 replications each year. Wilt counts were secured by cutting all stalks in each row and recording as infected all that showed internal discoloration. The table reveals that for any one year the variation in percentage of infection is so slight that no indication of resistance can be deduced for any variety tested

[4] Loc. cit.

TABLE 1.—*Actual wilt infection percentages, 1928–1931, on 18 varieties of cotton*

Variety	Average percentage infected			
	1928	1929	1930	1931
Cleveland 54	59.6	75.4	58.75	16.25
Miller 610	65.5	84.2	53.00	16.25
Lightning Express	68.7	85.4	34.00	17.25
Dixie Triumph	61.8	80.3	42.75	.....
Super Seven	47.5	79.1	18.25	.....
D. & P. L. 4–8	.....	66.3	23.50	13.25
Missdel No. 2	.....	88.1	47.50	11.75
D. & P. L. 4128A–21–32	71.0	75.7	.....	.....
Delfos 910	74.2	89.1	.....	.....
Express 121	57.7	.....	.....	17.50
Willis Triumph	.....	77.6	37.75	.....
D. & P. L. 5242A–310–46	.....	62.9	25.00	.....
Cook 1010	.....	81.6	38.75	.....
D. & P. L. 6	.....	82.3	22.75	.....
D. & P. L. 10	.....	.....	33.00	16.25
Express 121–41077	.....	.....	29.00	10.00
Missdel No. 3	.....	.....	34.75	21.75
Missdel No. 1	.....	.....	34.75	23.75
Average infection for above varieties	63.25	78.69	35.55	16.40
Average infection total number tested	62.82	80.00	35.53	17.66

or, at least, not on the basis of actual wilt infection counts at the end of the season.

In spite of the fact that the disease was discovered quite late in the fall of 1930, a brief scouting trip was made through a number of counties located chiefly in the Mississippi Delta to secure data regarding its distribution and prevalence. Collections of diseased cotton stalks were made from 21 properties located in 11 counties. Seven of these yielded cultures of the Verticillium, 10 yielded Fusarium only, and the other 4 gave neither Fusarium nor Verticillium. On account of the late date at which the collections were made many of the stalks collected were practically dead and contaminating saprophytic organisms had attained such a foothold that they outgrew and obscured the cultures of organisms that were responsible for the internal discolorations.

The counties in which Verticillium wilt was located in this brief survey were Bolivar, DeSoto, Tunica, and Washington. All of these are located in the Mississippi Delta and, in fact, immediately adjacent to the Missis-

sippi River. The soil in all cases was a heavy, sedimentary loam of the type usually termed gumbo.

In 1931 this survey was extended. County agents, Smith-Hughes teachers, State Plant Board inspectors, and other agricultural workers throughout the State were solicited to send in specimens of cotton plants showing symptoms of wilt. Internal discolorations of the woody tissues of the stem and taproot were in most cases taken as the diagnostic symptoms. Two or 3 brief scouting trips by the writers served to supplement somewhat this cooperative survey carried on by correspondence. All specimens received or collected were cultured immediately by the junior writer.

The total number of collections sent in by cooperators was 69. Twenty more were made by the writers, making a total of 89 in 1931. Each collection consisted of from 5 to 10 or more plants and each plant was cultured in a single Petri dish. Thirteen of the 89 collections cultured yielded cultures of Verticillium only, 73 showed Fusarium alone, and 3 yielded both Fusarium and Verticillium. Verticillium, therefore, was present in 16 and Fusarium in 76 of the 89 collections made. The map, figure 1, shows the distribution of these infections in the State.

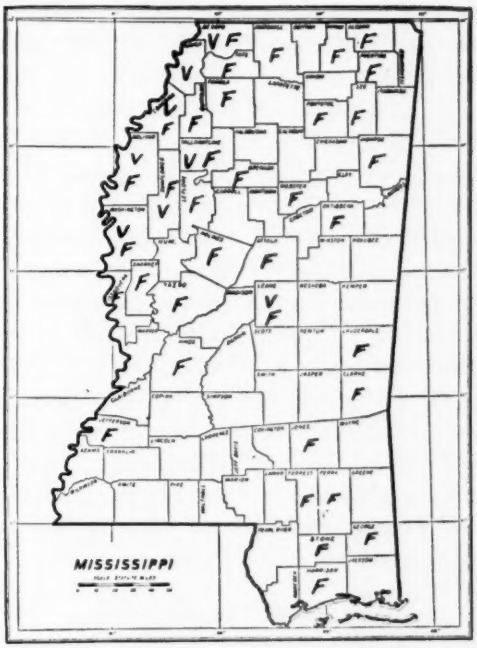

FIG. 1. Distribution of *Fusarium vasinfectum* and *Verticillium alboatrum* as revealed by the survey. F, Fusarium. V, Verticillium.

The collections cultured represented 37 of the 82 counties in Mississippi. Fusarium cultures were secured from plants from 36 of these 37

counties and Verticillium from 8. The total number of plants from which cultures of either Fusarium or Verticillium were secured was 706. Numerous others yielded no cultures of either or were overgrown with other contaminating organisms and were discarded. Six hundred and twenty-six of the 706 plants yielded Fusarium and 80 gave cultures of Verticillium. The counties in the State in which Verticillium is now known to occur are DeSoto, Tallahatchie, Bolivar, Sunflower, Coahoma, Tunica, Washington, and Leake. All of these are located in the Delta, with the single exception of Leake, which is in the central part of the State.

The infection in Leake County occurs on rich, loamy soil in river-bottom land. One of those in Tallahatchie County is in the hill section outside the limits of the Delta but is on bottom land of a rich, loamy nature. The others are all on typical Delta soils, mostly of the gumbo type. So far, the disease has not been found on any of the lighter or sandy soils of the State. Sherbakoff (5) states that he has found it only on the gumbo river-bottom soils of Tennessee and Arkansas. Rudolph (7), speaking of Verticillium in general as it occurs on a large number of host plants, states that opinion is sharply divided as to which soil types are most conducive to the development of the disease and the propagation of the fungus. He states, however, that in California Verticillium hadromycosis, incidentally on crops other than cotton, has been observed in severe epidemic form on soils varying from a light, sandy loam to a heavy clay and to adobe types. Bewley (3, 4) considers clay soils as favorable to the development of the disease on tomatoes. Van der Lek (8) also regards clay as well as sedimentary soils as favorable to the propagation of the fungus.

Observations made in Mississippi, though too few in number and superficial in character, tend to indicate that the heavier sedimentary and alluvial soils are more favorable to the disease than are the lighter, sandier types. This is, in fact, rather definitely indicated in the experimental plots at the Delta Branch Experiment Station on which the disease was first discovered in the State. The soil in one limited area near the central part of the plot is lighter in color and texture than the remainder and contains a larger proportion of sand in its composition. On this area the cotton during the last 2 years was observed to be somewhat shorter and of less vigorous growth and the percentage of plants showing infection was also considerably lower than on the heavier soils on either side.

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BLIGHT OF PEPPERS IN FLORIDA CAUSED BY PHYTOPHTHORA CAPSICI

GEORGE F. WEBER

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During the winter-growing season of 1930–31, large, bearing pepper plants of the Ruby King and California Wonder varieties growing in the Homestead area were found affected with a blight caused by *Phytophthora capsici* Leonian. This is the first time that this fungus has been collected in Florida and the second report of its collection in continental United States. The infected areas were small and confined to one section of the field, apparently resulting from the use of diseased seedlings originating from infected seed. The pathogen was isolated and identified through comparisons with Phytophthora species occurring in Florida, with a culture of the original species supplied by Leonian, and through artificial inoculations. This identification was verified by Tucker,[1] who had previously collected and determined this parasite from Puerto Rico. In addition to the establishment of the occurrence and pathogenicity of this parasite on peppers in Florida, the purpose of this paper is to present certain information concerning the symptoms of the disease in the field in addition to those given by Leonian,[2] as it occurred in New Mexico.

THE DISEASE

Symptoms. Diseased pepper plants in Florida showed infected stems, branches, fruit, and leaves. Infected stems were often girdled at the soil line, which resulted in a sudden wilting and death of the entire plant. The girdling lesions extended from slightly below the soil line to an inch or more up the stem at the time the plant wilted. They consisted of dark-green water-soaked bands, which later dried brown. The upper line of demarcation between the killed and living tissue was distinct. The width of the girdling band on the stems aboveground was greater when the point of infection was farther from the soil line. The entire tops of young plants were usually invaded by the time the plant died, while the tops of older plants were not invaded by the fungus, probably because the woodiness of the stems apparently retarded the spread of the fungus. When one branch was invaded the fungus, almost without exception, advanced to the primary stem and eventually killed the entire top.

[1] Tucker, C. M. Taxonomy of the genus Phytophthora De Bary. Missouri Agr. Exp. Sta. Res. Bul. 153. 1931. (See pp. 100–101.)

[2] Leonian, L. H. Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. nov. Phytopath. 12: 401–408. 1922.

On the leaves, the spots were at first small, circular or irregular in shape, and appeared scalded (Fig. 1). Later, they enlarged, became dried and bleached to a light-tan color, and were of a papery consistency. These areas eventually cracked and in some instances fell away from the unaffected tissue. No macroscopic evidence of the parasite was observed on the surface of affected leaf tissue. Entire leaves killed by these local lesions have not been observed, although extensive dead areas were common.

The fruit was infected through the stem ends by the advance of the fungus from stem lesions through the peduncle. Pod infections originating otherwise have not been observed. The peduncles were always discolored before macroscopical evidence of fruit invasion took place. The progress of the fungus through the fruit was determined by the dark-green,

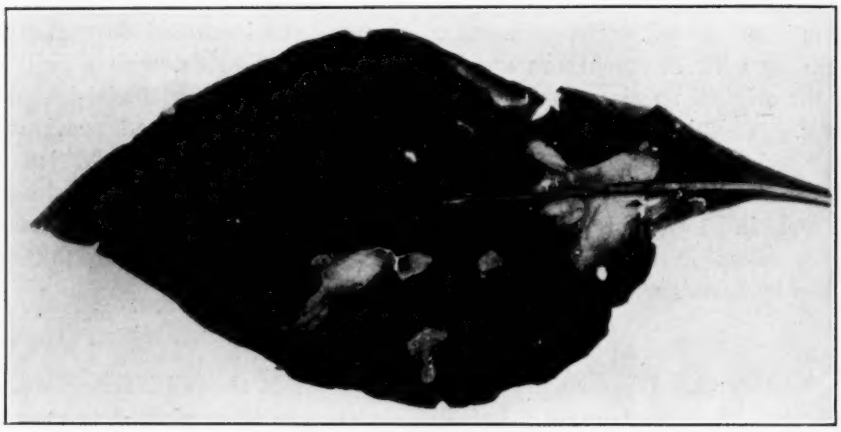

FIG. 1. Lesions on pepper leaf resulting from natural infection by *Phytophthora capsici*.

water-soaked appearance of the invaded cells in contrast to the light-green color of the uninvaded ones. The development of the fungous hyphae and fruiting structures on the surface of the pods lagged behind the advancing margin of disease from a few mm. to 1½ cm. The short conidiophores grow out through the epidermis in compact clumps and produce the conidia. After several days the entire pepper pod was invaded and covered with fruiting structures of the fungus. Following this stage the pod dried out rapidly and remained attached to the plant in mummied form (Fig. 2).

CULTURE OF THE ORGANISM

The parasite was isolated from stems, leaves, and fruit. It did not produce sporangia abundantly in culture, but the oospores were plentiful. Cultures of the originally described *Phytophthora capsici* and of *P. terres-*

tria Sherb.[3] were grown on various media simultaneously with the organism isolated from peppers in Florida. Always, the cultures from pepper were comparable and were distinct from *P. terrestria* Sherb.

INOCULATIONS

Uninjured pepper plants growing in the greenhouse and in field plots were inoculated by spraying with a water suspension of sporangia, and 54 per cent infection was obtained in the greenhouse and 19 per cent infection out of doors. All infected plants showed stem, branch, or leaf lesions.

FIG. 2. Pepper pods showing 3 stages of the disease caused by *Phytophthora capsici* in the field. Most recently infected pod is on the right.

Other pepper plants growing in the greenhouse were inoculated with sections, 4 mm. square, of hard poured-agar media overgrown by the fungus mycelium. The inoculum was placed fungus side to the plant in the fork of the first branches, some of which had been injured previously. After 30 hours' incubation in a moist chamber the plants were placed on a greenhouse bench where the disease became evident 3 days after inoculation. *Phytophthora capsici* and the *Phytophthora* sp. isolated from peppers in Florida produced the disease in 100 per cent of the trials on both injured and uninjured pepper plants (Fig. 3). The check plants and those inoculated with *P. terrestria* did not become diseased.

[3] *Phytophthora terrestria* Sherb. considered the same as *P. parasitica* Dastur, both having been considered as synonyms of *P. omnivora* De Bary. Leonian, L. H. Physiological studies on the genus Phytophthora. Amer. Jour. Bot. 12: 444–498. 1925.

FIG. 3. Pepper plants inoculated with *Phytophthora capsici* from Florida. Check plant at the left.

Green pepper pods were surface-sterilized and inoculated in the laboratory and incubated in glass moist chambers. They were inoculated by placing on the sterilized and aseptically clipped end of the peduncle a small square of hard potato agar covered with the fungus, placing the mycelium side in contact with the plant tissue. Infection took place rapidly and the pods inoculated with the organisms from pepper developed similar disease symptoms that were indistinguishable from those on naturally infected pods. In both cases the organisms were reisolated, and they compared favorably with each other and the original cultures. The pods similarly inoculated with *P. terrestria* and the checks did not become diseased (Fig. 4).

DISCUSSION AND CONCLUSIONS

The description of the disease of peppers caused by *Phytophthora capsici* in New Mexico is not entirely comparable with the symptoms of the disease observed in Florida. Some variation may be accounted for because of different kinds or varieties of peppers, the symptoms developing in New Mexico being described on Chile peppers, while in Florida the disease appeared on Ruby King and California Wonder varieties. In Florida, the leaves were infected and portions of them were killed, whereas leaf infection was not mentioned in connection with the disease in New Mexico. In Florida, it was observed that all fruit infection occurred at the stem end

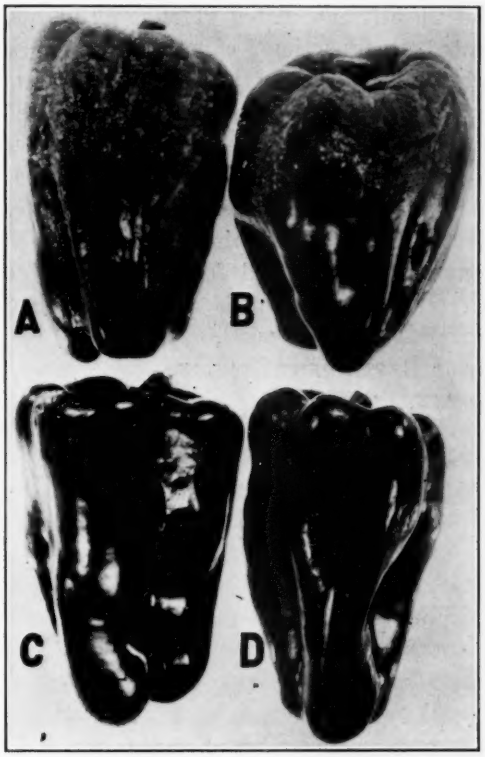

FIG. 4. Diseases of pepper pods resulting from artificial inoculations. A. *Phytophthora capsici*, from New Mexico. B. *P. capsici*, isolated from diseased plants in Florida. C. *P. terrestria*. D. Sterilized water (check).

as a result of the advance of the fungus from the branches through the peduncle. The fruit infection always advanced from the stem end to the blossom end of the pods, uniformly on all sides. The linear mode of advancement of the disease as described in New Mexico was not seen. In Florida, attacked pods always became completely overgrown by the parasite in contrast to frequent indefinite halts of advancement in the disease in New Mexico. The difference in symptoms may be accounted for largely by the differences in relative humidity during the respective growing seasons. Since these climatological data were not given in connection with the description of the disease in New Mexico, definite comparisons cannot be made. The relative humidity is generally high in Florida, especially in the Homestead section during the winter growing season. It may be that differences in relative humidity will account for the difference in the symptoms of the disease as described by Leonian for naturally infected

field plants and artificially inoculated greenhouse plants, this possibly replacing his suggested "mass-action" effect.

The fungus causing a blight of peppers in Florida was isolated and identified as *Phytophthora capsici*, previously described as causing a disease of Chile peppers in New Mexico. The diseases resulting from artificial inoculation of pepper plants with cultures of the New Mexico and Florida organisms were indistinguishable, whereas peppers inoculated at the same time with *P. terrestria* remained healthy.

This fungus, previously reported from continental United States but once, is herewith reported from Florida for the first time.

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SYNERGISM IN A BACTERIAL DISEASE OF HEDERA HELIX

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In the spring of 1931, several plants of the common, large-leaf type of English ivy, *Hedera helix* L., affected with a leaf spot were received by the Department of Plant Pathology at Cornell University for diagnosis. The plants were representative of a large shipment from Georgia consigned to a retail store in New York State. Microscopic examination revealed the presence of bacteria within the diseased tissues. Isolations were made from typical spots and, in certain instances, 2 distinct types of bacteria were obtained from a single lesion. Both of the organisms produced round, yellow colonies on beef-extract agar. They differed in that one type had a slower rate of growth and was somewhat lighter in color. Inoculation experiments demonstrated that the slower-growing organism (Type I) was pathogenic. The other organism, which grew more rapidly and was darker (Type II), proved to be nonpathogenic when used alone.

A bacterial disease of English ivy has been the subject of three different publications from Europe. In 1894, Lindau[1] described a disease of this plant and attributed its cause to a bacterium. He made no attempt, however, to culture the organism or prove its pathogenicity. Arnaud,[2] in 1920, reported what appeared to be the same disease from France and named the associated organism *Bacterium hederae*, n. sp. A description of the organism was omitted, however, and proof of its pathogenicity was again lacking. One year later, Killian[3] obtained infection on ivy with a bacterium and reported the pathogen's reaction on several media. White[4] first reported the presence of the disease in this country and in a later publication[5] presented a complete description of the symptoms exhibited by affected plants together with considerable information in regard to various pathological phases of the problem.

From a comparison with the symptoms described in earlier reports, it was evident that we were dealing with the same disease. On the leaves, the first symptom of infection consists of a small water-soaked area about the

[1] Lindau, G. Der Epheukrebs. Ztschr. Pflanzenkr. 4: 1–3. 1894.

[2] Arnaud, G. Une maladie bactérienne du lierre (*Hedera helix* L.). Compt. Rend. Acad. Sci. (Paris) 171: 121–122. 1920.

[3] Killian, C. Une maladie bactérienne du lierre. Compt. Rend. Soc. Biol. (Paris) 84: 224–226. 1921.

[4] [White, R. P.] Bacterial leafspot of *Hedera helix*. New Jersey Agr. Exp. Sta. Nursery Dis. Notes 3 (6): 4. 1930. (Mimeographed.)

[5] [White, R. P.] Diseases of *Hedera helix*. New Jersey Agr. Exp. Sta. Nursery Dis. Notes 4 (1): 1–4. 1931. (Mimeographed.)

point of entrance. As the spots increase in size, the lesions develop dark brown to black centers. In the final stages, and especially with mature leaves, the water-soaked margins around the spots disappear and the centers crack as they become dry. A distinct red purple margin is usually visible at this time. Natural infections on the leaf petioles and stems were not present on our material, but, according to White,[6] such infections do occur.

In preliminary experiments to determine which of the 2 organisms was responsible for the disease, a series of plants was inoculated with Type I alone, another series with Type II alone, and, as a matter of interest, a third series with Types I and II together. The inoculations were made by placing the bacteria in small wounds made on the foliage by means of needle punctures. It was interesting to note that infection occurred first on those plants that had been inoculated with the mixture of Types I and II. The initial symptoms were visible in 6 days, and it was a week later before the plants inoculated with Type I alone showed evidences of infection. The wounded checks and the series inoculated with Type II alone gave negative results.

This experiment has been repeated 3 times with similar results. The disease acceleration obtained by mixing the 2 types of organisms can be shown by measurements of the diameters of spots on plants inoculated under similar conditions with either one or both of the two organisms. A set of these data are presented in the following table. Plants and leaves of the same relative age were used and the measurements were made 20 days after the plants had been inoculated.

Diameter of spots in mm.	Number of spots			
	Inoculum Type I	Inoculum Type II	Inoculum Types I and II	Wounded check
0.0	0	54	1	54
1.0	16	0	0	0
1.5	13	0	0	0
2.0	20	0	8	0
2.5	2	0	16	0
3.0	3	0	18	0
3.5	0	0	0	0
4.0	0	0	11	0

In this particular experiment, the spots did not increase rapidly in size, due to cool temperatures prevailing in the greenhouse. It was very evident, however, that the bacteria representative of Type II had a distinct

[6] See footnote 5.

accelerating action upon the pathogen, Type I. In other experiments, where the greenhouse temperatures have been higher, the difference in size of the spots was more striking.

It should be pointed out that the effect of the accelerator was not lasting. Approximately 4 weeks from the date of inoculation, the infections caused by Type I alone were similar in size and appearance to those resulting from Types I and II together. Furthermore, the addition of the accelerator at the time of inoculation did not have any significant effect on the relative percentages of infection. In all experiments thus far conducted, we have obtained practically 100 per cent infection with Type I alone and with Types I and II together, while all inoculations with the accelerator alone have given negative results.

Suggestions as to the exact nature of this case of synergism are not offered in this preliminary report, but the following observations are of interest. In 4 attempts to reisolate the pathogen and the accelerating organism from artificially produced lesions where both organisms had been used it was found that from the small spots the 2 organisms appeared in the plates in approximately equal numbers. From the large lesions the accelerating organism greatly predominated.

Since a complete description of the pathogen causing the leaf spot of ivy has never been published, the following work on its cultural characteristics was undertaken and is presented here. The specific name "*hederae,*" as suggested by Arnaud, is used, although this investigator did not have the organism in pure culture. There seems to be no doubt, however, that he was working with the same disease, since his description of the symptoms is identical with ours. Furthermore, the name *hederae* has crept into literature dealing with phytopathology and, to avoid future confusion, should be retained. A complete description of the accelerating organism also is given, but without a name. It appears, however, to be similar to the epiphyte frequently referred to in European literature as *Bacterium herbicola aureum.* It should be stated that this accelerating organism is much more active biochemically than the pathogen.

Phytomonas hederae is a slender rod, Gram-negative and motile by means of a single polar flagellum. Spores are not formed. Its average size is 2.13 μ by 0.6 μ. On beef-extract-agar slants growth is good, filiform and amber yellow; its consistency is watery to butyrous; milk is cleared and finally becomes alkaline; gelatin is liquefied; nitrates are not reduced; indol and hydrogen sulphide are not formed; ammonia is produced in peptone broth; the following carbohydrates are fermented but with no production of gas, dextrose, levulose, galactose, xylose, lactose, sucrose, glycerol, and the sodium salts of acetic, citric, lactic, malic, and succinic

acid; and the following are not fermented, arabinose, rhamnose, maltose, salicin, starch, cellulose, and the sodium salt of formic acid. The organism is a faculative anaerobe.

The accelerating organism is a short rod, at times oval with a central granule, Gram-negative and motile by means of one to two polar flagella. There are no spores. Its average size is 1.7 μ by 0.9 μ. On beef-extract-agar slants growth is good, filiform, and antimony yellow; its consistency is butyrous; milk at first turns alkaline but later becomes neutral or slightly acid; nitrates are reduced; indol and hydrogen sulphide are not formed; ammonia is produced in peptone broth; the following carbohydrates are fermented rapidly without gas, dextrose, levulose, galactose, arabinose, xylose, rhamnose, maltose, sucrose, glycerol, mannitol, salicin, and the sodium salts of acetic, citric, lactic, malic, and succinic acids; and the following are not fermented, lactose, starch, cellulose, and the sodium salts of formic and tartaric acid. The organism is a definite facultative anaerobe.

Further work on pathological phases of the problem is in progress.

SUMMARY

A case of synergism in a bacterial disease of English ivy is described. From certain leaves, 2 distinct types of bacteria were isolated. The organisms differed slightly in color and rate of growth on beef-extract agar. The slower-growing bacterium proved to be the pathogen, while the other organism was nonpathonegic when used alone. When the 2 organisms were combined for the purpose of inoculation, the nonpathogen was shown to have a distinct accelerating action upon the disease complex.

A complete description of the pathogen, *Phytomonas hederae*, is given. The accelerating organism also is described, but without a name.

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PHYTOPATHOLOGICAL NOTES

Bacterial and Fungous Flora in Certain Sulphur Fungicides.—In conducting certain toxicity tests with various sulphur fungicides, there was experienced a degree of contamination on seeded plates that could not be explained through any procedure of conventional technique. As a result of this situation, attention was given to the possible contamination that certain sulphur fungicides might be carrying. Representative samples of 14 various sulphur fungicides were collected that included, for the most part, mixtures of straight elemental sulphur, modified sulphurs, which, in addition to the elemental sulphur, may contain hydrated lime and wettable agents, and suspended sulphurs. Qualitative procedure, such as the inoculation of a tube of bouillon with a small loopful of the fungicide, followed by a 5-day incubation period and then seeding on agar plates will provide evidence in contaminations of dominant organisms. In order to determine the probable contaminations from a quantitative standpoint, the technique used for soil-flora studies was adopted. This procedure included the use of 1 gm. of the fungicide with 100 cc. of sterile water in a sterile flask. This mixture (A) was placed on a shaker for 30 minutes and additional dilutions of 10^{-4} (B) and 10^{-5} (C) were made from the original mixture. From each of these 3 dilutions, 1 cc. was withdrawn from A and 1/10 cc. from B and C with a sterile pipette and seeded with melted agar media in sterile plates. Only 3 types of media were selected for this work, namely, nutrient agar, nutrient glucose agar, and Ashby's agar. It was found that these media were favorable for growth of certain contaminating organisms but would not represent a range of media favorable for isolating all possible contaminating organisms, especially in those sulphur fungicides that are modified with casein and other wettable agents. Some of the fungicidal mixtures showed bacteria present in numbers exceeding 1,000,000 per gm., while fungi were found in numbers up to 30,000 per gm. These results strongly indicate that the contaminations are much higher than would normally be expected for natural air-exposed materials.

The bacterial flora for the most part represented species of the *Bacterium fluorescens* group, and the fungi were species of Aspergillus and Penicillium. No study was made as to the possible presence of anaerobic or gas-producing forms of bacteria. That contaminations should be encountered is natural when one considers the various steps in the preparation and handling for retail trade of various forms of dry sulphur fungicides. Particularly in those types of modified sulphurs where caseinate and similar substitutes are added to improve the wetting and physical properties there is found a greater contamination than is met with in samples of elemental sulphur. Samples of modified sulphurs 2 years of age showed a

heavier contaminating flora than did fresh samples. Suspended sulphurs, especially from by-products in gas manufacturing, showed the heaviest bacterial flora, with over 1,000,000 per gm. of material. When the original dilutions of the fungicides were allowed to stand 4 weeks and then seeded, the number of contaminating organisms invariably increased. No attempt was made to determine whether the organisms found were either beneficial or detrimental to the efficiency of the fungicide.

These facts are not presented as the result of a complete investigation of the subject but principally for the purpose of raising 3 questions. (1) Is a contaminating flora in certain sulphur fungicides beneficial or detrimental? (2) How much contamination should be permitted? (3) Whether it is not pertinent, in view of facts reported, that precaution should be taken in determining the purity of certain sulphur fungicides before using them for toxicity tests or determining their fungicidal or bactericidal action.—J. F. ADAMS, Agricultural Experiment Station, University of Delaware, Newark, Delaware.

Notes on Phyllosticta rabiei on Chick Pea, II.—Some years after Trotter[1] published the results of his excellent work on the fungus *Phyllosticta rabiei* (Pass.) Trotter, Labrousse,[2] and more recently the writer,[3] published partial confirmation of Trotter's studies. The writer pointed out that only a small percentage (less than 5 per cent) of the conidia were septate, and those only faintly. While the writer agreed with Trotter that the fungus was closer to Phyllosticta than to Ascochyta, it was pointed out by him that the genetic connection with Ascochyta was possibly closer than Trotter suspected. It must be borne in mind, however, that nonseptate conidia very frequently form cross walls just previous to germination.

Recently, Labrousse[4] carried this study still further and proposed *Ascochyta rabiei* (Pass.) nov. comb. He pointed out that *A. pinodella* L. K. Jones has a high percentage of nonseptate spores and that he was in agreement with Trotter and the writer that the chick-pea fungus is somewhere genetically connected with the leguminous Ascochytae.

The difficulty of placing the border-line fungi in some of these genera of *Fungi Imperfecti* is well known and the *Phyllosticta-Ascochyta-Stagonospora* complex is one of the most troublesome. While the writer realizes

[1] Trotter, A. La "rabbia" o "anthracnosi" del cece ed il suo produttore. Riv. Patal. Veg., n.s. **9**: 105–114. 1918.

[2] Labrousse, L. L'anthracnose du pois-chiche (*Cicer arietinum*). Rev. Path. Vég. et Ent. Agr. **17**: 174–177. 1930.

[3] Sprague, R. Notes on *Phyllosticta rabiei* on chick pea. Phytopath. **20**: 591–593. 1930.

[4] Labrousse, F. L'anthracnose du pois-chiche (2e note). Rev. Path. Vég. et Ent. Agr. **18**: 226–231. 1931.

that technically Labrousse's position is well open to support, he feels that Trotter's diagnosis is the more conservatively taken. If we are to place every Phyllosticta with a small percentage of septate spores in the genus Ascochyta it will be necessary, for the sake of consistency, to place some physiologic forms of *Ascochyta pisi* Lib. in the genus Stagonospora or, at least, in the doubtful genus Stagonosporopsis Died. We would then have the awkward situation of a certain physiologic form of a fungus or even a growth form recognized as a full-fledged species in an entirely different genus.

As to the appropriateness of the genus Phyllosticta for species that attack other organs in addition to leaves, it may be noted that such fungi as *Phyllosticta solitaria* Ell. and Ev., which attacks twigs and fruits as well as leaves, have long stood undisturbed in the genus Phyllosticta. In an herbaceous plant, such as the chick pea, there is even less reason for attaching any taxonomic value to leaf versus stem parts.

The writer does not consider *Ascochyta pinodella* L. K. Jones as a parallel case, because, very frequently, collections of this fungus show close to 100 per cent of the spores septate, while in mature specimens of *Phyllosticta rabiei*, 96 to 98 per cent of the spores are nonseptate. That is, *A. pinodella* is typically uniseptate, while *P. rabiei* is predominatingly nonseptate. The routine worker should have no particular difficulty in placing a collection of *A. pinodella* in its accepted niche, but it would require considerable study for him to see any connection of *Phyllosticta rabiei* with Ascochyta. In order to avoid further confusion in this the writer pleads that Trotter's combination of *P. rabiei* (Pass.) Trotter be retained.—RODERICK SPRAGUE, United States Department of Agriculture, at Oregon State Agricultural College, Corvallis, Oregon.

Color Variations in Bacterial Plant Pathogens.—In 1930 the writer published a note on a white strain of *Aplanobacter michiganense* E. F. Sm., the cause of bacterial canker of tomato. Since that time white variants have been observed in three other yellow bacterial pathogens, namely, *Bacterium campestre* (Pam.) E. F. Sm., *Bact. vesicatorium* Doidge, and *Bact. cucurbitae* Bryan. In all three cases, the variation was discovered in old beef-agar stock cultures, in the form of wedges in otherwise normally yellow surface growth. In the case of *Bact. vesicatorium* the white strain was also isolated directly from typically spotted fruit from Mexico in 1931 and proved to be as infectious as the yellow strain, producing typical spots on fruits and leaves. The variations in cultures have occurred only in an occasional tube. Cultures of sister colonies of the same age and held under identical conditions remained normal.

In the case of each of the three pathogens mentioned above, the white strain was separated by means of poured plates. Transfers from single white colonies were replated, giving only white colonies. Transfers from these colonies were then used for inoculation of suitable hosts in the hothouse. The white *Bacterium campestre* was as virulent as the yellow strain from the same culture, *i.e.*, the one from which the white was separated. *Bacterium vesicatorium* was also as infectious as the normal strain, but in the case of *Bact. cucurbitae* the white strain was decidedly less virulent than the yellow one.

The white color, in the case of all these bacteria, persists in the various culture media and comes out pure when reisolations are made from inoculated plants. Reversion to yellow has occurred in only an occasional culture in stocks held for months in the ice box under conditions similar to those under which the original variation appeared.

The strains of each organism have been compared in the most common culture media: beef agar and broth, beef gelatin, potato cylinders, litmus milk, and nitrate broth, without discovering any differences aside from color.

It seems probable that other yellow pathogens, in both nature and in culture, produce color variants that have not been recognized as such. Since *Aplanobacter michiganense* also has a pink strain, this color as well as the white should be borne in mind when working with "normally" yellow organisms.—MARY K. BRYAN, Department of Agriculture, Washington, D. C.

A New Form of Oat Stem Rust from a Barberry Area.—A new physiologic form of oat stem rust (*Puccinia graminis avenae*) was identified in two collections made in 1930 in a barberry area near Jefferson, Wisconsin. Many barberry bushes have been found in this area in the past, and at the time the collections were made there were barberries in the vicinity, over 4,000 bushes and seedlings being found in the county in 1930. Inasmuch as oat fields near the bushes were rusted and a general infection had not yet appeared in the fields of surrounding territory, it seems logical to assume that the new form came from near-by barberries. The new form, which has been called "form 10," has not yet been found elsewhere. Unsuccessful attempts were made to isolate it again from Jefferson County collections in 1931.

The two collections of form 10 were cultured in the greenhouse for more than 8 months and both have behaved consistently on the differential varieties of oats. Form 10 attacks Richland (C. I. 787), which is resistant to forms 1, 3, and 7, also forms 2 and 5, the most prevalent forms of oat stem rust in the United States. While form 4 and 6 also infect Richland

heavily, White Tartar (C. I. 551) is very susceptible to these forms but resistant to form 10; and Joanette Strain (C. I. 2660), resistant to form 4 and susceptible to form 6, reacts indeterminately to form 10 (infection type X), the reaction resembling that of this variety to form 5 as described by Stakman, Levine, and Bailey.[1]

Since it has been shown experimentally that new forms of wheat stem rust are produced by hybridization on the barberry and since there is evidence that they actually are being produced in nature,[2] it appears probable that new forms of oat stem rust may originate in the same way. All the evidence points to the fact that form 10 probably originated through hybridization or segregation on barberries, but there is a question whether it has become established. (Cooperative investigations between the Division of Barberry Eradication and the Division of Cereal Crops and Diseases, United States Department of Agriculture, and the Minnesota Agricultural Experiment Station.)—RALPH U. COTTER, University Farm, St. Paul, Minn.

[1] Stakman, E. C., M. N. Levine, and D. L. Bailey. Biologic forms of *Puccinia graminis* on varieties of *Avena* spp. Jour. Agr. Res. **24**: 1013–1018. 1923.

[2] Stakman, E. C., Lee Hines, Ralph U. Cotter, and M. N. Levine. Physiologic forms of *Puccinia graminis* produced on barberries in nature. (Abst.) Phytopath. **22**: 25. 1932.